Longitudinal analysis of morphological and functional changes in intermediate age-related macular degeneration

Doctoral thesis

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List of abbreviations

Anti-VEGF	Anti-vascular endothelial growth factor
AMD	Age-related macular degeneration
AO-SLO	Adaptive optics scanning laser ophthalmoscopy
AREDS	Age-Related Eye Disease Study
ART	Automatic Real Time
ARVO	Association for Research in Vision and Ophthalmology
BCVA	Best-corrected visional acuity
BM	Bruch's membrane
CFP	Color fundus photography
CNV	Choroidal neovascularization
cSLO	Confocal scanning laser ophthalmoscopy
dB	Decibel
ETDRS	Early treatment diabetic retinopathy study
ELM	External limiting membrane
FAF	Fundus autofluorescence
FCP	Fundus-controlled perimetry
FRET	Full retinal thickness
FU	Follow up
GA	Geographic atrophy
HRF	Hyperreflective foci
HRS	Hyperreflective specks

iAMD	Intermediate age-related macular degeneration
ILM	Internal limiting membrane
IRET	Inner retinal thickness
IS/OS	Inner-outer segment junction
LLVA	Low-luminance visual acuity
LogMAR	Logarithm of the minimum angle of resolution
nAMD	neovascular age-related macular degeneration
NIR	Near-infrared reflectance
OCT	Optical coherence tomography
OPL	Outer plexiform layer
ONL	Outer nuclear layer
PR	Photoreceptor
PWS	Point-wise sensitivity
PWSL	Point-wise sensitivity loss
RPE	Retinal pigment epithelium
RPEDC	Retinal pigment epithelium-drusen complex
SD	Standard deviation
SD-OCT	Spectral-domain optical coherence tomography
SE	Standard error

1. English summary

1.1 Introduction

1.1.1 Background: age-related macular degeneration and its clinical significance

Age-related macular degeneration (AMD) is the leading cause for an irreversible damage of central vision in elderly individuals, especially in industrialized countries. Currently, an estimated 30-50 million people worldwide are affected by AMD and the prevalence is expected to increase even further due to increased life expectancy and an ageing global population (Colijn et. al., 2017; Wong et. al., 2014). The progressive nature of AMD significantly impacts the quality of life, leading to difficulties in daily activities such as reading, driving, and recognizing faces.

AMD is clinically characterized by a gradual decline in visual function, with patients in the early stages primary experiencing reduced visual acuity under low-light or dark-adapted conditions. As the disease progresses, visual impairment worsens, eventually leading to a central scotoma and profound vision loss in advanced cases. The growing prevalence of AMD underscores the urgent need for targeted therapies, particularly for the early and intermediate stages of the disease, where no approved treatment options currently exist to prevent further progression.

1.1.2 Classification and pathophysiology of AMD

AMD is typically classified based on clinical and imaging findings, with the Age Related Eye Diseases (AREDS) group classifies AMD categorizing the disease into early, intermediate, and late stages according to different fundus lesions and associated vision loss. (Mitchell et. al., 2018; Lim et. al., 2012).

Early AMD is characterized by the presence of medium-sized drusen (63-125 µm) without pigmentary abnormalities. At this stage, patients may remain asymptomatic, although subtle functional changes may already be occuring.

Intermediate AMD (iAMD) is defined by the presence of large drusen (>125 µm) with or without central hyperpigmentation. This stage is associatedc with more noticeable visual impariments, particularly under low-light conditions.

Late AMD is further subdivided into central geographic atrophy (GA) and neovascular age-related macular degeneration (nAMD), also known as choroidal neovascularization (CNV). GA is characterized by progressive atrophy of the different anatomical layers including the retinal pigment epithelium (RPE), photoreceptors and choroidocapillaris, leading to irreversible central vision loss. In contrast, nAMD is marked by the abnormal proliferation of choroidal blood vessels, which may lead to fluid leakage, hemorrhages, and rapid deterioration of central vision.

1.1.3 Current challenges in AMD treatment

Over the past two decades, significant progress has been made in the treatment of nAMD, particularly with the introduction of anti-vascular endothelial growth factor (anti-VEGF) therapies, which have been widely used to effectively suppress CNV progression and reduce associated vision loss. Additionally, ongoing research into gene therapies and novel pharmacological approaches offers hope for further advancements in the management of nAMD (Volz and Pauly, 2015).

Despite these advancements, there are currently no effective therapies yet available for early or iAMD to halt the progression loss of GA. Furthermore, an increasing prevalence of AMD in industrialized countries will further intensify the demand for target-oriented therapies. This treatment gap highlights the urgent need for novel therapeutic strategies that can slow AMD progression and preserve visual function before irreversible damage occurs.

1.1.4 Advances in imaging and functional assessment in the early stages of AMD

Multimodal imaging techniques have revolutionized our ability to detect and monitor subtle retinal changes in AMD. Spectral-domain optical coherence tomography (SD-OCT) has become a key tool for assessing retinal morphology, allowing for detailed

visualization of drusen characteristics, RPE integrity, and outer retinal atrophy. Recent studies have identified structural biomarkers that may serve as early indicators of AMD progression. For instance, Schlanitz et al. revealed that the integrity of the photoreceptor layers was significantly related to the shape and diameter of drusen in early and iAMD (Schlanitz et. al., 2019), while Echols et al. further discovered that the presence of hyperreflective lesions within the neurosensory retina, so called hyperreflective foci (HRF), are associated with reduced rod- and cone-mediated retinal function, further implicating an indicator of disease progression (Echols et. al., 2020).

In addition to structural imaging, functional assessments such as low-luminance visual acuity (LLVA) have been reported to be reduced in iAMD patients and hence may be used as an indicator for central-cone-mediated retinal function under reduced illumination (Chandramohan et. al., 2016). However, LLVA lacks the precision needed for detailed retinal functional assessment, making it less suitable for tracking localized disease progression. Functional deficits in iAMD are not limited to visual acuity loss but also involve impairments in dark adaption and contrast sensitivity. More accurate methods are required to measure impairment of localized rod- or cone-mediated retinal function.

To address these limitations, fundus-controlled perimetry (FCP, also called microperimetry) has emerged as a powerful tool for assessing retinal sensitivity under mesopic and scotopic conditions. Unlike standard visual acuity tests, FCP provides topographic information on localized retinal function, making it invaluable for understanding disease progression.

1.1.5 Importance of scotopic and mesopic functional testing in AMD

Under normal physiological conditions, a central scotoma corresponding to the rod-free fovea (approximately to the central 1°-2°) is present in dim-light conditions. Around the central 5°-6° with a high density of rods, however, a greater sensitivity is observed under dark adaption (Crossland et. al., 2011). Two-color scotopic and mesopic FCP allows for partially selective testing of rod function by measuring rod sensitivity following dark adaptation. By performing scotopic and mesopic tests of retinal function, early

dysfunction of the central macular region has been found in eyes with iAMD (Steinberg et. al., 2015; Welker et. al., 2018). Furthermore, in a 12-month follow-up study this loss of mesopic and scotopic sensitivity has been reported to proceed even further (Nguyen et. al., 2018).

Moreover, investigations have described a correlation between microstructural and functional changes such as the thickness of the inner-outer segment junction (IS/OS) layer, the outer nuclear layer (ONL) and the retinal pigment epithelium-drusen complex (RPEDC) (Landa et. al., 2011; Saßmannshausen et. al., 2018). Landa et al. demonstrated a significant loss of IS/OS layer associated with poor retinal sensitivity, while Sassmannshausen et al. discovered that a reduction of both, mesopic and scotopic FCP, were associated with an increasing thickness of the total retina and the RPEDC as well as a decrease of the ONL and the photoreceptor (PR) segment thickness. Similarly, Wu et al. found a significant relationship between the thickness of the RPEDC layer and presence of HRF with a mesopic fundus-controlled sensitivity loss over a 12-month time frame (Wu et. al., 2016).

1.1.6 Aims of this thesis

Despite significant advances in our understanding of AMD pathogenesis, the longitudinal relationship between microstructural retinal changes and progressive mesopic and scotopic function decline remains poorly understood in iAMD. Based on the assumption that rod-degeneration precedes cone-degeneration in AMD pathogenesis, a stronger association of retinal layer thickness changes with scotopic than mesopic sensitivity losses would be expected (Sadda et. al., 2018).

Therefore, the aim of this study is to examine the relationship between morphological changes in retinal layer thickness and visual functional changes, as assessed by mesopic and scotopic FCP, in iAMD patients with large drusen over a 3-year follow-up period. By integrating multimodal structural imaging with functional assessments, this study aims to provide novel insights into the mechanisms underlying AMD progression and contribute to the development of more precise prognostic biomarkers for earlier intervention.

1.2 Materials and methods

1.2.1 Study design, ethical approval, and study population

This prospective and non-interventional study was conducted at the Department of Ophthalmology, University of Bonn, Germany, over a period of three years, from October 2016 to March 2019.

The study was approved by the local ethics committee (Ethik-Kommission, Medizinische Fakultät, Rheinische Friedrich-Wilhelms-Universität; Lfd. Nr.125/14) and complied with the tenets of the Declaration of Helsinki. Before participation, all subjects provided written informed consent after receiving a detailed explanation of the study procedures, risks, and objectives.

The study included two groups: AMD patients diagnosed with iAMD in at least one eye; age-matched healthy control participants with no evidence of retinal disease. Participants were recruited from the outpatient department of the University eye hospital Bonn through routine clinical visits and referrals. Individuals who met the inclusion criteria and consented to participate underwent a standardized baseline ophthalmologic examination and imaging protocol. For healthy controls, only a single baseline visit was required. For AMD patients, three additional follow-up visits were scheduled annually over a three-year period, during which multimodal imaging and functional testing were repeated.

To ensure a homogenous study population, strict eligibility criteria were applied. For the AMD group, the inclusion criteria were: the diagnosis of iAMD (large drusen >125 µm, with or without pigmentation abnormalities in either eye) in at least one eye, according to the international classification system (Ferris III et. al., 2013) (**Fig. 1**); best-corrected visual acuity (BCVA) better than logarithm of the minimum angle of resolution (logMAR) 0.2; clear ocular media sufficient for high-quality imaging; stable fixation for reliable functional testing and multimodal imaging.

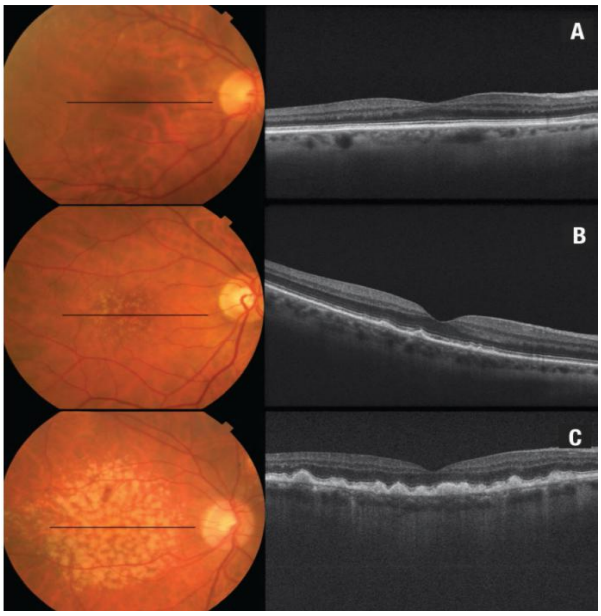

Fig. 1: Fundus photography with corresponding SD-OCT shows no AMD (A), early AMD (B) and iAMD (C). A: no signs of AMD, with no visible drusen or pigmentation abnormalities; B: signs with medium drusen (63-125 µm), but without pigmentation abnormalities thought to be related to AMD; C: signs with large drusen (>125 µm) or with pigmentation abnormalities associated with at least medium drusen. Images of late AMD (neovascular AMD or GA) are not shown here. (Seddon et. al., 2019).

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; GA, geographic atrophy.

For the healthy control group, the inclusion criteria were: No evidence of AMD or any other retinal disease; no history of ocular surgery, except for uncomplicated cataract surgery; age-matched to the AMD participants.

Participants from both groups were excluded if they met any of the following conditions: the presence of significant anterior segment disease (e.g., severe cataract, corneal opacities); any retinal pathology aside from AMD (e.g., diabetic retinopathy, retinal vein occlusion, hereditary retinal diseases); previous intraocular surgery aside from cataract surgery; history of anti-VEGF therapy for neovascular AMD or other retinal conditions; presence of neurological conditions affecting vision (e.g., optic neuropathy, stroke); systemic diseases that could affect visual function, such as uncontrolled diabetes mellitus.

1.2.2 Comprehensive ophthalmic examination

All participants underwent a standardized comprehensive ophthalmologic assessment, which included visual function tests, slit-lamp examination as well as fundus biomicroscopy and multimodal retinal imaging assessments. Visual function tests were consist of BCVA assessment by the early treatment diabetic retinopathy study (ETRDS) chart and LLVA testing under standardized reduced illumination. Anterior segment and fundus examination were performed to rule out ocular pathologies. Multimodal imaging assessments included color fundus photography (CFP), fundus autofluorescence (FAF). SD-OCT for retinal layer segmentation and thickness analysis. Functional testing like FCP to assess retinal sensitivity in mesopic and scotopic conditions.

For AMD patients, all these assessments were repeated annually during the three-year follow-up period.

1.2.3 Multimodal imaging and morphological assessment

High-resolution SD-OCT imaging was performed using the Heidelberg Spectralis HRA+OCT (digital image resolution 768 x 768 pixels; Heidelberg Engineering, Germany), a device equipped with a confocal scanning laser ophthalmoscope (cSLO) and SD-OCT technology.

The standardized retinal imaging protocol consisted of CFP, FAF (exc λ=488 nm, em λ=500-800 nm, minimum 15 frames), single horizontal and vertical combined cSLO+SD-OCT scan across the fovea (30°, automatic real time (ART) minimum 9 scans) and raster SD-OCT scan (30°x 25°, ART minimum frames, 61 B-scans, distance 120 µm) focusing on the fovea center. Retinal imaging was performed at baseline and annually over three years for AMD patients.

For the morphological assessment semi-automatic retinal layer segmentation was performed by the Heidelberg Eye Explorer (Heidelberg Engineering, Heidelberg, Germany). Automatic retinal layer segmentation was reviewed in each scan and manually corrected if indicated. Retinal layer segmentation was defined as follows: the full retina layer running from the internal limiting membrane (ILM) to the Bruch’s

membrane (BM), the inner retinal thickness (IRET) ranging from the ILM to the outer plexiform layer (OPL) and the ONL running from the OPL to the external limiting membrane (ELM). Please also see **Fig. 2** for details on retinal layer segmentation.

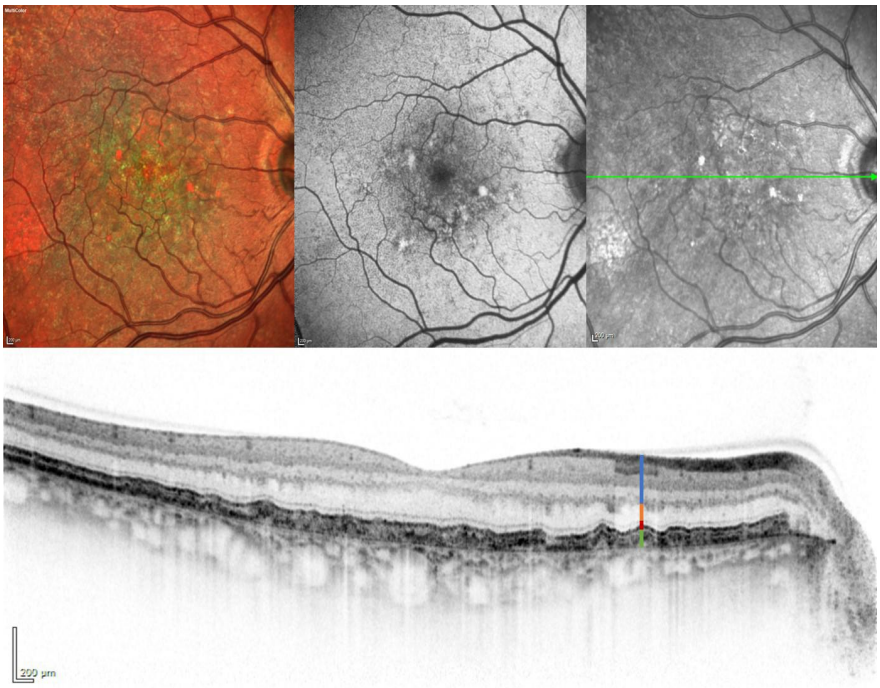

Fig. 2: Multimodal retinal imaging including multicolor(upper left), blue light autofluorescence (upper middle) and SD-OCT raster scan (upper right). Retinal layers (lower) of the total retina (blue line +orange line +green line) were segmented including the inner retina (blue line), the ONL (orange line), the IS/OS (red line) and the RPEDC (green line) for the assessment of different retinal thicknesses.

1.2.4 Functional analysis

Functional analysis was performed by using FCP (Nidek MP-1S; Nidek Technologies, Padova, Italy) for retinal sensitivity testing. For patients and control subjects mesopic (Goldmann size III, 200 ms 4-2 staircase threshold strategy, background luminance 1.27cd/m^2, 3° radius and 1-pixel fixation ring) and scotopic (Goldmann size V) FCP testing was performed with a 56-stimuli point test grid (10°x 10°) centered on the fovea. AMD participants had a baseline and three follow-up examinations during the 3-year period, while the control group only received the baseline examination. A representative

example of a 73-year-old female patient with localized mesopic and scotopic FCP changes during the follow-up time is shown in **Fig. 3**.

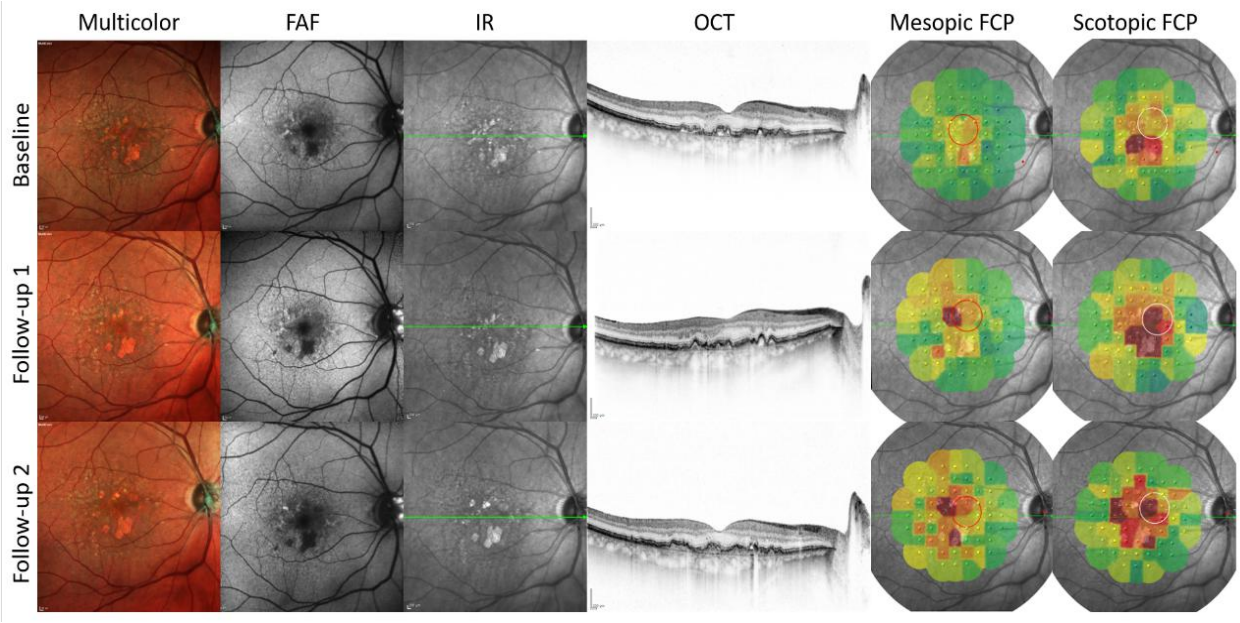

Fig. 3: 73-year-old female with iAMD. Performed were multicolor, fundus autofluorescence (FAF), near-infrared reflectance (NIR), SD-OCT, mesopic and scotopic from baseline to follow-up 2. A more pronounced decline of scotopic than mesopic sensitivity was detected in the FCP examination (right) as well as an enlargement of central large drusen. A progressive decline of retinal sensitivity at different follow-up visits was detected, highlighted by orange and red fields in FCP testing.

1.2.5 Structure-function correlation

For structural-functional assessment, retinal sensitivity values of mesopic and scotopic testing were correlated point-to-point to retinal thickness data deriving from the SD-OCT imaging. Therefore FCP data were registered and aligned to a SD-OCT en-face image using nonlinear affine transformation based on retinal vessel bifurcations. Then, corresponding retinal thickness data at the precise site of the stimuli for mesopic and scotopic testing were generated for each retinal layer.

1.2.6 Statistical analysis

Statistical analyses were performed using the software of the R programming language for baseline analysis as well as the longitudinal structure-function analysis. Accordingly, FCP and SD-OCT data were standardized to z-scores to fully account for the topographic dependence of mean values and inter-individual variability. For the analysis of the correlation of retinal layer thickness changes and follow-up time on mesopic and scotopic function, mixed effect models were applied. Two-sided $p<0.05$ was considered statistically significant.

1.3 Results

1.3.1 Population demography

59 eyes of 54 patients with iAMD and 27 eyes from 27 healthy controls were included in this prospective study. Among AMD eyes, 38 participated at first-year follow-up time, 25 at second-year follow-up time and 14 at third-year follow-up time. The mean standard error (SE) age of AMD and healthy participants was 71.72 ± 8.97 years (range, 45-85) and 64.65±8.86 years (range, 50-81). Additionally, BCVA and LLVA were measured at each follow-up visit. No overall significant intergroup variation was found in visual acuity or lens status. A summary of demographic characteristics is outlined in **Tab. 1**.

Tab. 1: Characteristics of Study Subjects

	Patients (n=59)	**Controls (n=27)**
Age, mean±SD	71.72 (8.97)	64.65 (8.86)
Visual Acuity (LogMAR)		
BCVA	0.1	0.0
LLVA	0.4	0.3
LLVA Deficit	0.3	0.3
Lens Status, n (%)		
Phakic	40 (60.80)	23 (85.19)
Pseudophakic	19 (32.20)	4 (14.81)

Abbreviations: SD, standard deviation.

1.3.2 Retinal thickness analysis

At baseline, the retinal thickness of the ONL was reduced by -0.49 SD [- 0.70; - 0.28], while the RPEDC layer showed a thickening of 3.22 SD [2.27; 4.17] in patients with iAMD (p<0.001). This corresponds in terms of thickness deviation to -4.94 µm for the ONL and +7.93 µm for the RPEDC. There were no statistically significant thickness changes at baseline for the full retinal thickness (FRET), IRET and IS/OS retinal layers.

During the follow-up period, an increase in RPEDC thickness was revealed with +0.51 SD/year [0.28; 0.74] (1.23 µm/year), while a decrease in the thickness of the ONL and IS/OS with -0.03 SD/year [-0.07; 0.0] (-0.32 µm/year) and with -0.34 SD/year [-0.40; -0.27] (-0.76 µm/year), respectively (p<0.001). For the FRET and the IRET layers, no significant thickness changes over time were exhibited. Details of the retinal thickness changes are shown in **Tab. 2**.

Tab. 2: Retinal thickness changes at baseline and follow-up visit

Parameter	Estimate[1] (as Z Score)	95% CI	*P* Value	Estimate (as Thickness Deviation)
FRET thickness				
Baseline	0.07 SD	-0.14 to 0.27	0.481	1.83 µm
Follow-up	0.00 SD/year	-0.02 to 0.03	0.916	0.21 µm/yr
IRET thickness				
Baseline	-0.06 SD	-0.23 to 0.12	0.532	-1.43 µm
Follow-up	-0.00 SD/year	-0.02 to 0.02	0.945	0.01 µm/yr
ONL thickness				
Baseline	-0.49 SD	-0.70 to -0.28	**<0.001**	-4.94 µm
Follow-up	-0.03 SD/year	-0.07 to -0.00	**0.045**	-0.32 µm/yr
IS/OS thickness				
Baseline	0.10 SD	-0.14 to 0.33	0.401	0.21 µm
Follow-up	-0.34 SD/year	-0.40 to -0.27	**<0.001**	-0.76 µm/yr
RPEDC thickness				
Baseline	3.22 SD	2.27 to 4.17	**<0.001**	7.93 µm
Follow-up	0.51 SD/year	0.248 to 0.74	**<0.001**	1.23 µm/yr

[1]SD-OCT thickness values represent z-scores (number of normative SD from respective mean in normal eyes)

1.3.3 Assessment of retinal function

At baseline the mean pointwise retinal sensitivity loss (PWSL) was -1.67 decibel (dB) [-2.22; -1.12] (estimate [confidence interval]) for mesopic and -2.34 dB [-2.85; -1.84] for scotopic testing, respectively (p<0.001).

Over time, PWSL further decreased in eyes by -0.35 dB/year [-0.43; -0.28] for mesopic and increased by +0.20 dB/year [0.12; 0.29] for scotopic testing (p<0.001). **Tab. 3** displays a summary of the functional analysis. A representative patient example is illustrated in **Fig. 3** (for further details, see the results in the supplement).

Tab. 3: Changes in retinal sensitivity with scotopic and mesopic FCP

Parameter	Pointwise Sensitivity Loss (dB)	95% CI	*P* Value
Scotopic FCP			
Baseline (intercept)	-2.34	-2.85~-1.84	**<0.001**
Change/year	+0.20	+0.12~0.29	**<0.001**
Mesopic FCP			
Baseline (intercept)	-1.67	-2.22~-1.12	**<0.001**
Change/year	-0.35	-0.43~-0.28	**<0.001**

1.3.4 Correlation between structural and functional outcome measures

Univariable linear mixed-effect models were applied to detect a possible correlation between retinal layer thickness changes and retinal sensitivity changes. There was no significant influence of the IRET and the IS/OS thickness on pointwise mesopic and scotopic sensitivity loss, whereas a significant association was observed between both types of sensitivity testing and the retinal thickness of the FRET, the ONL and the RPEDC layers (p<0.001).

In detail, regarding point-wise sensitivity (PWS) change for each normative SD of retinal layer thickening, the most noticeable correlation was exhibited for the ONL (+0.25 dB/SD $_{ONL}$ [0.20; 0.30]), followed by the FRET (-0.18 dB/SD $_{FRET}$ [-0.24; -0.11]), and the RPEDC thickness (-0.05 dB/SD $_{RPEDC}$ [-0.05; -0.04]), respectively p<0.001. A higher association

with scotopic PWS was revealed for the ONL (+0.48 dB/SD $_{ONL}$ [0.41; 0.54)], followed by the FRET (-0.20 dB/SD $_{FRET}$ [-0.27; -0.13]) and the RPEDC thickness (-0.05 dB/SD $_{RPEDC}$ [-0.06; -0.05]), respectively $p<0.001$. Moreover, follow-up time showed a significant association with mesopic and scotopic PWS loss, respectively ($p<0.001$).

The combined linear mixed-effect models revealed that the thickness of the ONL ($p<0.001$) and the RPEDC (p=0.015) layer were significantly associated with mesopic sensitivity loss, whereas the thickness of the FRET ($p<0.001$), IRET (p=0.001), ONL ($p<0.001$) and the IS/OS ($p<0.001$) also exhibited a significant correlation with scotopic sensitivity. Besides, follow-up time also remained a significant predictor for both mesopic and scotopic retinal function, respectively ($p<0.001$). A representative graph of the retinal layer thickness for each layer and follow-up time on mesopic and scotopic retinal sensitivity is provided in **Fig. 4.**

After considering potential floor and ceiling effects and excluding any PWS values less than 2 dB or over 18 dB, we discovered that estimates for mean PWS loss at baseline changed for mesopic and scotopic measures, respectively with -2.46 dB [-2.89; -2.04] and -2.56 dB [-2.96; -2.16]. Longitudinal estimates for change in retinal sensitivity remained similar with changes of -0.28 dB/year [-0.36; -0.20] for mesopic, and +0.13 dB/year [0.05; 0.21] for scotopic testing ($p<0.001$).

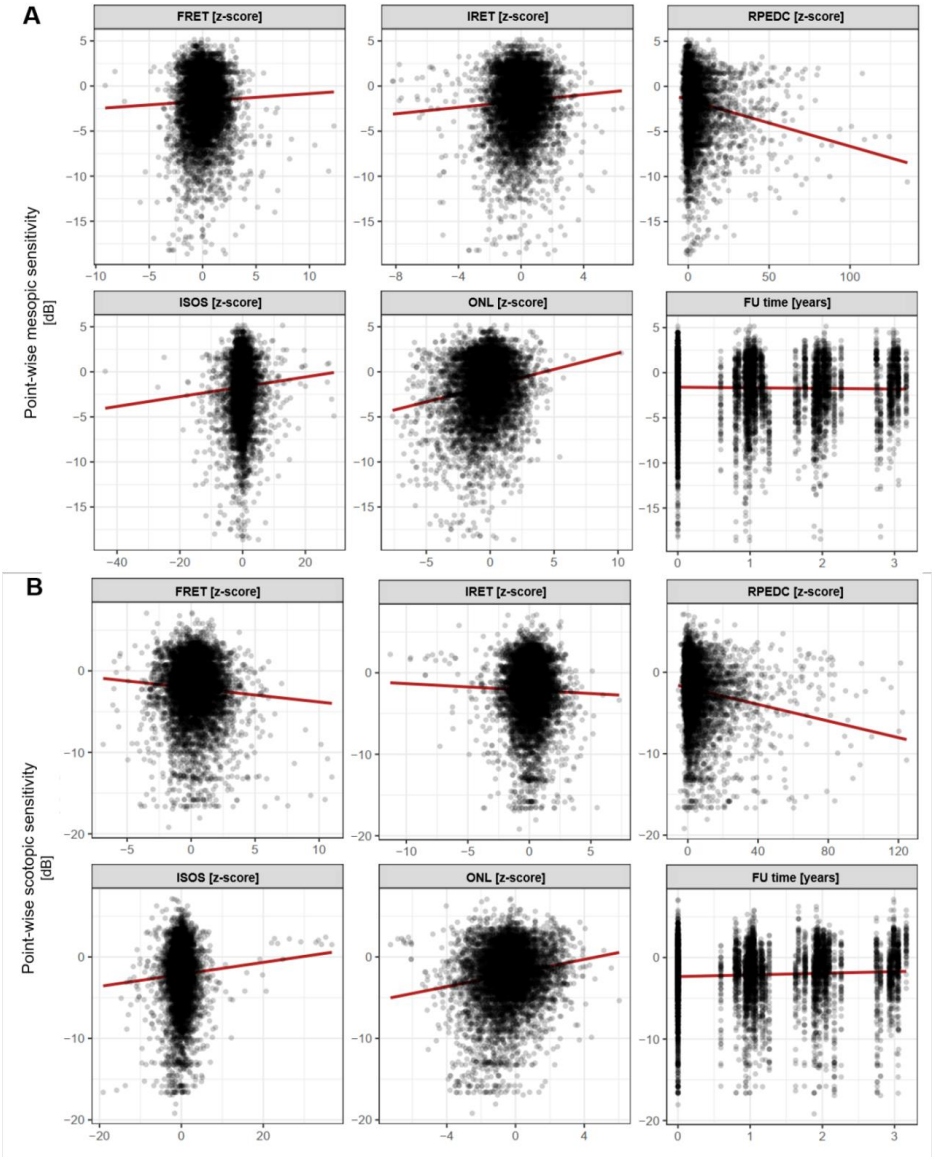

Fig. 4: A graphical illustration of the dependence of retinal layer thickness and follow-up time on mesopic and scotopic retinal FCP. Scatterplots showing the pointwise (A) mesopic and (B) scotopic retinal sensitivity (in decibels) in dependence of retinal thickness for FRET, IRET, RPEDC, photoreceptor IS/OS layer, ONL and dependence of follow up (FU) time. Thickness data were plotted in terms of SD of the normative mean (Z scores). Follow-up time was plotted in years. (Sassmannshausen et. al., 2021)

1.4 Discussion

1.4.1 Key findings and comparison with previous studies

In this study, we quantified retinal thickness changes, especially of the outer retina, and correlated these changes longitudinally to a loss of retinal function measured by FCP in eyes with iAMD. In detail, at baseline there was a significant thinning of the ONL as well as a significant thickening of the RPEDC layer. Within a 3-year follow-up period, we further observed a dynamic change of outer retinal layer thinning, which correlated to a localized loss of mesopic and even more pronounced loss of scotopic sensitivity. In addition, we displayed that there was an independent effect of follow-up time on retinal sensitivity.

Of note, our findings demonstrated a more pronounced decline of scotopic than mesopic retinal function being associated with retinal layer thickness changes within a longitudinal period in presence of large drusen. Histopathologic studies have shown that photoreceptor cell and RPE changes arise in the early stages of AMD, leading to functional changes of the RPE and subsequently to a reduced retinal rhodopsin content which is related to rod-specific cells (Curcio et. al., 1996). According to previous publications, Owsley et al. demonstrated that disturbances in rod-mediated but not cone-mediated dark adaptation were characteristic of early AMD by measuring both cone- and rod-mediated dark adaptation (Owsley et. al., 2007). Therefore, a possible explaining for our findings of RPEDC layer thickening and ONL thinning in iAMD could be due to the presence of drusen between RPE and Bruch’s membrane and consequently damage of photoreceptor outer segments, which cause decreased function of rod cells and thus a larger sensitivity loss in scotopic FCP testing compared to mesopic retinal function. Our results are also consistent with some recent clinical studies, which have described a higher vulnerability of rod than cone photoreceptors within AMD (Owsley et. al., 2007; Steinberg et. al., 2016). However, these studies did not correlate outer retinal thickness changes to retinal function within a longitudinal period.

In this study, it was recognized that subtle changes in the functional features occur in the intermediate stage of AMD. Our findings demonstrated that a stronger impairment of scotopic than mesopic FCP in eyes with large drusen being in line with previous report. The average baseline sensitivity decrease for mesopic in iAMD eyes was -1.67 dB [95% confidence interval: -2.22; -1.12], and -2.34 dB [-2.85; -1.84] for scotopic testing, respectively. Over time, mesopic sensitivity deviation stronger decreased by -0.35 dB/year being in line with findings by Nebbioso and coworkers (Steinberg et. al., 2015; Nebbioso et. al., 2014). Some researchers have reported mesopic sensitivity loss within a 12-month follow-up time, e.g. Wu et al. found an average decrease of -0.42 dB in eyes with iAMD and Hsu et al. discovered a mean loss of -3.0 dB (Wu et. al., 2015). To the best of our knowledge, this is the first longitudinal study of a 3-year period to have examined the natural progression of structure and function in iAMD eyes at multiple retinal locations.

In terms of retinal microstructural analysis at baseline, we have discovered a localized ONL thinning as well as a localized RPEDC thickening in AMD patients similar to prior studies (Nivison-Smith et. al., 2018; Pappuru et. al., 2011). Other studies further reported that retinal thickness changes of the RPE layer were supposed to be an early precursor to the formation of GA lesions (Sadigh et. al., 2013). Actually, the nature of these results is the same, as the disease progress, drusen might disappear while RPEDC thickness begins to decrease and RPE atrophy evolves. We have not observed that in our study possibly because our follow-up time is too short to observe the same phenomenon. Thus, further studies with longer follow-up periods are needed for possible confirmation.

Interestingly, we observed an unexpected trend where scotopic sensitivity showed slight improvement rather than continuous decline over the 3-year period, whereas we found a stronger reduction of scotopic than mesopic macular function at baseline. One potential explanation is the localized compensation mechanisms, such as adaptive changes in retinal circuitry or increased metabolic support from the RPE, which may temporarily stabilize function in certain areas. Another possibility is that our FCP testing protocol captured inherent variability in retinal sensitivity, which may not always reflect a purely linear decline. However, a longer follow-up period would be necessary to determine whether this trend persists or if scotopic function eventually deteriorates further. Future

studies with extended follow-up durations may clarify whether mesopic function loss follows the scotopic decline, as previously suggested.

Beyond our findings, other recent studies have explored additional biomarkers of disease progression in iAMD. Ferrara D et al. suggested that alterations in the ellipsoid zone integrity and interdigitation zone disruption on optical coherence tomography (OCT) could serve as early indicators of AMD progression, highlighting the potential for multimodal imaging to provide a more comprehensive assessment of structural changes (Ferrara D, 2017). Moreover, emerging evidence from adaptive optics scanning laser ophthalmoscopy (AO-SLO) studies has demonstrated that direct visualization of rod and cone photoreceptor mosaic disruptions may provide additional insights into the pathophysiology of iAMD (Wynne N, 2021). These findings suggest that integrating high-resolution imaging modalities with functional assessments such as FCP may enhance our understanding of disease progression in AMD.

1.4.2 Study limitations and future research directions

The limitations of our study included the relatively small sample size in each group and the obvious decrease of participants over time, as well as the fact that there were no follow-ups for control subjects limiting the statistical power of detecting subtle changes. Additionally, the absence of a control group for longitudinal comparison makes it difficult to differentiate disease-specific changes from normal aging-related retinal alterations. Future studies should include age-matched healthy controls and a larger cohort of iAMD patients to confirm whether observed changes in retinal thickness and FCP are solely attributable to AMD progression rather than age-related structural variations.

Another limitation is the inherent variability of functional testing. FCP measurements, although widely used, are subject to intra-individual variability, particularly in patients with AMD who may have difficulty maintaining fixation due to drusen-related visual disturbances. Future research should integrate emerging retinal imaging technologies, such as AO-SLO and hyperspectral imaging, to provide higher-resolution structure-function correlation analyses. These advanced imaging techniques may help detect

subtle microstructural changes before they manifest as significant functional impairments.

Furthermore, our study does not account for potential systemic and genetic risk factors that may influence AMD progression. Recent genome-wide association studies have identified several genetic variants associated with AMD susceptibility, including polymorphisms in the CFH and ARMS2/HTRA1 genes (Stradiotto E, 2022). Future research should investigate how these genetic factors interact with structural and functional changes over time, potentially paving the way for personalized risk stratification and treatment strategies.

1.4.3 Conclusion

In conclusion, this study provides a detailed longitudinal assessment of structure-function relationships in iAMD over a 3-year period. Our findings suggest that progressive thinning of the outer retina corresponds with localized scotopic and mesopic sensitivity losses, with scotopic impairment being more pronounced. These results reinforce the hypothesis that rod photoreceptor dysfunction precedes cone dysfunction in AMD and that monitoring outer retinal thickness changes may serve as a robust biomarker for disease progression. Future studies should focus on longer follow-up durations, larger sample sizes, and the integration of novel imaging and genetic analyses to further elucidate the mechanisms underlying AMD progression. This knowledge may ultimately aid in the development of targeted therapeutic interventions aimed at preserving retinal function in patients with the earlier stages of AMD.

1.5 Summary

Purpose: Longitudinal analysis of retinal thickness changes of mesopic as well as scotopic retinal function in patients with iAMD.

Methods: Fifty-nine eyes of 54 AMD patients with large drusen (>125 µm) underwent annual mesopic and scotopic FCP testing and SD-OCT scans over a 3-year time frame. 27 eyes of age-matched healthy control participants were only examined at baseline. Structural analysis assessed the thickness of each retinal layer, particularly the outer retinal layer.

Results: The average PWS decrease for baseline in iAMD patients was -1.67 dB [95% confidence interval: -2.22; -1.12] for mesopic and -2.34 dB [-2.85; -1.84] for scotopic testing, respectively. During 3 years of follow-up time, the sensitivity deviation in patients was decreased by -0.35 dB/year [-0.43; -0.28] under mesopic and increased by +0.20 dB/year [0.12; 0.29] under scotopic testing, respectively ($p<0.001$). At baseline, the mean relative thickness of ONL was significantly reduced by -0.49 SD [-0.70; -0.28] in eyes with iAMD compared to normal eyes, while the mean localized thickness of RPEDC was significantly increased by +3.22 SD [2.27; 4.17]. Over time, the further variation of RPEDC thickness was +0.51 SD/year, -0.03 SD/year for ONL, and -0.34 SD/year for the inner and outer photoreceptor segments, respectively ($p<0.001$). Furthermore, structure-function analysis revealed that the change of ONL and RPEDC thickness was associated with both mesopic and scotopic FCP sensitivity ($p<0.001$). Follow-up time displayed an independent effect on retinal sensitivity, respectively ($p<0.001$).

Conclusion: Longitudinal FCP sensitivity changes in mesopic retinal function were associated with progressive quantifiable degeneration of the outer retina in iAMD, suggesting that these structure-function parameters may serve as surrogate markers in future clinical trials examining the early stages of AMD.

1.6 References for the English summary

Chandramohan A, Stinnett SS, Petrowski JT, Schuman SG, Toth CA, Cousins SW, and Lad EM. Visual function measures in early and intermediate age-related macular degeneration. Retina 2016; 36: 1021

Colijn JM, Buitendijk GH, Prokofyeva E, Alves D, Cachulo ML, Khawaja AP, Cougnard-Gregoire A, Merle BM, Korb C, and Erke MG. Prevalence of age-related macular degeneration in Europe: the past and the future. Ophthalmology 2017; 124: 1753-1763

Crossland MD, Luong VA, Rubin GS, and Fitzke FW. Retinal specific measurement of dark-adapted visual function: validation of a modified microperimeter. BMC ophthalmology 2011; 11: 1-7

Curcio CA, Medeiros NE, and Millican CL. Photoreceptor loss in age-related macular degeneration. Investigative ophthalmology visual science 1996; 37: 1236-1249

Echols BS, Clark ME, Swain TA, Chen L, Kar D, Zhang Y, Sloan KR, Mcgwin Jr G, Singireddy R, and Mays C. Hyperreflective foci and specks are associated with delayed rod-mediated dark adaptation in non-neovascular age-related macular degeneration. Ophthalmology Retina 2020; 11: 1059-1068

Ferrara D SR, Louzada Rn, Novais Ea, Collins Gk, Seddon Jm. Optical Coherence Tomography Features Preceding the Onset of Advanced Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci. 2017; 58: 11

Ferris Iii FL, Wilkinson C, Bird A, Chakravarthy U, Chew E, Csaky K, Sadda SR, and Committee BIFMRC. Clinical classification of age-related macular degeneration. Ophthalmology 2013; 120: 844-851

Landa G, Su E, Garcia PM, Seiple WH, and Rosen RB. Inner segment–outer segment junctional layer integrity and corresponding retinal sensitivity in dry and wet forms of age-related macular degeneration. Retina 2011; 31: 364-370

Lim LS, Mitchell P, Seddon JM, Holz FG, and Wong TY. Age-related macular degeneration. The Lancet 2012; 379: 1728-1738

Mitchell P, Liew G, Gopinath B, and Wong TY. Age-related macular degeneration. The Lancet 2018; 392: 1147-1159

Nebbioso M, Barbato A, and Pescosolido N. Scotopic microperimetry in the early diagnosis of age-related macular degeneration: preliminary study. Biomed Res Int 2014; 2014: 671529

Nguyen CT, Fraser RG, Tan R, Caruso E, Lek JJ, Guymer RH, and Luu CD. Longitudinal changes in retinotopic rod function in intermediate age-related macular degeneration. Investigative ophthalmology visual science 2018; 59: AMD19-AMD24

Nivison-Smith L, Wang H, Assaad N, and Kalloniatis M. Retinal thickness changes throughout the natural history of drusen in age-related macular degeneration. Optometry Vision Science 2018; 95: 648-655

Owsley C, Mcgwin Jr G, Jackson GR, Kallies K, and Clark M. Cone-and rod-mediated dark adaptation impairment in age-related maculopathy. Ophthalmology 2007; 114: 1728-1735

Pappuru RR, Ouyang Y, Nittala MG, Hemmati HD, Keane PA, Walsh AC, and Sadda SR. Relationship between outer retinal thickness substructures and visual acuity in eyes with dry age-related macular degeneration. Investigative ophthalmology visual science 2011; 52: 6743-6748

Sadda SR, Guymer R, Holz FG, Schmitz-Valckenberg S, Curcio CA, Bird AC, Blodi BA, Bottoni F, Chakravarthy U, and Chew EY. Consensus definition for atrophy associated with age-related macular degeneration on OCT: classification of atrophy report 3. Ophthalmology 2018; 125: 537-548

Sadigh S, Cideciyan AV, Sumaroka A, Huang WC, Luo X, Swider M, Steinberg JD, Stambolian D, and Jacobson SG. Abnormal thickening as well as thinning of the photoreceptor layer in intermediate age-related macular degeneration. Investigative ophthalmology visual science 2013; 54: 1603-1612

Saßmannshausen M, Steinberg JS, Fimmers R, Pfau M, Thiele S, Fleckenstein M, Holz FG, and Schmitz-Valckenberg S. Structure-function analysis in patients with intermediate age-related macular degeneration. Investigative ophthalmology visual science 2018; 59: 1599-1608

Sassmannshausen M, Zhou J, Pfau M, Thiele S, Steinberg J, Fleckenstein M, Holz FG, and Schmitz-Valckenberg S. Longitudinal Analysis of Retinal Thickness and Retinal Function in Eyes with Large Drusen Secondary to Intermediate Age-Related Macular Degeneration. Ophthalmol Retina 2021; 3: 241-250

Schlanitz F, Baumann B, Sacu S, Baumann L, Pircher M, Hitzenberger CK, and Schmidt-Erfurth UM. Impact of drusen and drusenoid retinal pigment epithelium elevation size and structure on the integrity of the retinal pigment epithelium layer. British Journal of Ophthalmology 2019; 103: 227-232

Seddon JM, Dossett JP, Widjajahakim R, and Rosner B. Association between perifoveal drusen burden determined by OCT and genetic risk in early and intermediate age-related macular degeneration. Investigative ophthalmology visual science 2019; 60: 4469-4478

Steinberg JS, Fitzke FW, Fimmers R, Fleckenstein M, Holz FG, and Schmitz-Valckenberg S. Scotopic and Photopic Microperimetry in Patients With Reticular Drusen and Age-Related Macular Degeneration. JAMA Ophthalmol 2015; 133: 690-697

Steinberg JS, Saßmannshausen M, Fleckenstein M, Fimmers R, Oishi A, Holz FG, and Schmitz-Valckenberg S. Correlation of partial outer retinal thickness with scotopic and mesopic fundus-controlled perimetry in patients with reticular drusen. American journal of ophthalmology 2016; 168: 52-61

Stradiotto E AD, Fossati G, Raimondi R, Sorrentino T, Tripepi D, Barone G, Inforzato a, Romano Mr. Genetic Aspects of Age-Related Macular Degeneration and Their Therapeutic Potential. Int J Mol Sci 2022; 32: 13280

Volz C and Pauly D. Antibody therapies and their challenges in the treatment of age-related macular degeneration. European Journal of Pharmaceutics Biopharmaceutics 2015; 95: 158-172

Welker SG, Pfau M, Heinemann M, Schmitz-Valckenberg S, Holz FG, and Finger RP. Retest Reliability of Mesopic and Dark-Adapted Microperimetry in Patients With Intermediate Age-Related Macular Degeneration and Age-Matched Controls. Invest Ophthalmol Vis Sci 2018; 59: AMD152-AMD159

Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng C-Y, and Wong TY. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. The Lancet Global Health 2014; 2: e106-e116

Wu Z, Ayton LN, Luu CD, and Guymer RH. Longitudinal changes in microperimetry and low luminance visual acuity in age-related macular degeneration. JAMA Ophthalmol 2015; 133: 442-448

Wu Z, Cunefare D, Chiu E, Luu CD, Ayton LN, Toth CA, Farsiu S, and Guymer RH. Longitudinal associations between microstructural changes and microperimetry in the early stages of age-related macular degeneration. Investigative ophthalmology visual science 2016; 57: 3714-3722

Wynne N CJ, Duncan JI. Promises and pitfalls of evaluating photoreceptor-based retinal disease with adaptive optics scanning light ophthalmoscopy (AOSLO). Prog Retin Eye Res 2021; 83: 100920

2. Publication

Longitudinal analysis of retinal thickness and retinal function in eyes with large drusen secondary to intermediate age-related macular degeneration.

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Longitudinal Analysis of Retinal Thickness and Retinal Function in Eyes with Large Drusen Secondary to Intermediate Age-Related Macular Degeneration

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Purpose: To assess the longitudinal association between outer retinal microstructure and mesopic as well as scotopic retinal sensitivity in patients with drusen secondary to intermediate age-related macular degeneration (iAMD).

Design: Prospective, longitudinal natural history study.

Participants: Fifty-nine eyes of 54 patients with large drusen (> 125 μm) associated with iAMD and 27 age-matched healthy control eyes.

Methods: Participants underwent spectral-domain OCT and both mesopic and scotopic fundus-controlled perimetry (FCP). Annual follow-up visits were performed over a 3-year period.

Main Outcome Measures: Pointwise correlation of retinal sensitivity stimuli to corresponding standardized (*Z* score) pointwise retinal thickness. Linear mixed-effect models were applied to analyze longitudinally the association of pointwise retinal thickness changes, follow-up time, or both with retinal function.

Results: At baseline, mean pointwise sensitivity in patients was reduced by −1.67 dB (95% confidence interval [CI], −2.22 to −1.12) for mesopic and by −2.34 dB (95% CI, −2.85 to −1.84) for scotopic testing compared with controls with a pointwise sensitivity change of −0.35 dB/year (95% CI, −0.43 to −0.28) for mesopic and +0.20 dB/year (95% CI, 0.12−0.29) for scotopic testing, respectively ($P < 0.001$). Retinal thickness analysis in patients revealed a significantly thinner outer nuclear layer (ONL) by −0.49 standard deviation (SD; 95% CI, −0.70 to −0.28 SD) and a significant thicker retinal pigment epithelium−drusen complex (RPEDC) by +3.22 SD (95% CI, 2.27−4.17 SD) at baseline, respectively ($P < 0.001$). During follow-up, retinal thickness thickened further by +0.51 SD/year (RPEDC) and thinned by −0.03 SD/year (ONL; $P = 0.045$) and −0.34 SD/year (inner and outer photoreceptor segments) in patients, respectively ($P < 0.001$). Structure-function analysis showed a significant association of the ONL and the RPEDC thickness change with both types of FCP sensitivity testing ($P < 0.001$). Besides, follow-up time had a significant (independent) effect on mesopic and scotopic retinal sensitivity ($P < 0.001$).

Conclusions: The longitudinal structure−function correlation demonstrated a progressive quantifiable degeneration of the outer retina in iAMD associated with photoreceptor dysfunction. Because longitudinal sensitivity changes could not be explained by structural changes alone, an unmet need remains for additional refined parameters on retinal structure to predict retinal function. *Ophthalmology Retina 2020;■:1−10 © 2020 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

Supplemental material available at www.ophthalmologyretina.org.

Despite of the breakthrough of anti−vascular growth factor (VEGF) therapy in the neovascular late stage of the disease, age-related macular degeneration (AMD) remains a leading cause of irreversible blindness and visual impairment in industrial countries.[1−4] Although anti−vascular growth factor treatment is well established as an effective method to retard progressive vision loss in late AMD stages, no effective interventions are available yet for early or intermediate stages of disease. The presence of large soft drusen (> 125 μm), also described as hallmarks of intermediate AMD, are as high-risk factors for progression to a late stage of disease as choroidal neovascularization and geographic atrophy development.[5−9]

With the introduction of high-resolution imaging in retinal diseases, a number of studies have reported microstructural changes associated with drusen secondary to AMD.[10] In this context, Rogala et al[11] demonstrated a localized thinning of the retinal pigment epithelium

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(RPE)−photoreceptor layer and of the outer nuclear layer thickness up to 32% over drusen compared with nonaffected eyes. Similar results were found by Schumann et al[12] with a reduction of photoreceptor thickness by 27.5% overlying drusen compared with healthy control participants, while inner retinal thickness remained unaltered.

Besides morphologic thickness changes within AMD disease, progressive visual impairment beyond preserved best-corrected visual acuity in early and intermediate AMD has been reported in various studies. Previous results from a patient-reported outcome assessment demonstrated increased visual impairment under dim-light or dark-adapted light conditions that are in accordance with recently shown reduced low-luminance visual acuity (LLVA) in early and intermediate AMD.[13−17] To overcome the limitations of current applied functional tests in clinical settings, for example, best-corrected visual acuity assesses only high-contrast photopic vision at the preferred retinal locus, establishment of more robust and reliable functional end points is warranted for future clinical interventional trials.[18,19]

In this context, fundus-controlled perimetry (FCP; also called 'microperimetry') may serve as a complementary functional examination tool to measure and monitor mesopic and scotopic retinal sensitivity spatially.[20] In a previous study, we demonstrated that both mesopic and scotopic sensitivity are reduced in intermediate AMD, whereas losses are more pronounced within the central 4° in contrast to more peripheral areas of the retina.[21] Regarding longitudinal studies on structural and functional changes within disease progress, only limited data are available. A progressive reduction of mesopic sensitivity within 12 months was shown by Vujosevic et al,[22] whereas decreased rod intercept time and reduction of scotopic retinal sensitivity within 1 year was reported recently by Nguyen et al.[23] In a further detailed structure-function analysis, Wu et al[24] demonstrated a significant association of RPE−drusen complex (RPEDC) thickening and presence of hyperreflective foci with mesopic fundus-controlled sensitivity loss. However, detailed longitudinal analysis of structural changes in association with scotopic FCP in intermediate AMD over an extended observational period have not been reported. Based on the assumption that rod degeneration precedes cone degeneration in AMD, scotopic sensitivity would be expected to exhibit a stronger association with retinal layer thicknesses than mesopic sensitivity.[21,25] Therefore, the aim of this study was to assess the extent of outer retinal degeneration in patients with intermediate AMD and investigate the association between retinal microstructure and mesopic as well as scotopic retinal sensitivity over a period of 3 years.

Methods

Participants

For participation in this prospective, noninterventional, observational natural history study, patients and age-matched healthy control participants were recruited at the Department of Ophthalmology, University of Bonn, Bonn, Germany. Patients were monitored in addition to the baseline visit by up to 3 annual visits between October 2016 and March 2019, whereas age-matched control participants were examined once at the baseline visit. The identical study protocol was applied in patients and control participants at baseline and each patient's follow-up visit. For inclusion, patients had to be diagnosed with intermediate AMD in the presence of large drusen (> 12 5μm) as shown by stereo biomicroscopy and near-infrared plus OCT imaging in either eye according to the international classification system by Ferris et al.[5] Any signs of late-stage AMD, including complete RPE and outer retinal atrophy, were defined as exclusion criteria.[25]

For control participants, age-matched healthy eyes without any signs of any ocular pathologic features were included. Furthermore, for both patients and control participants, only eyes with clear media, visual acuity of at least 0.2 logarithm of the minimum angle of resolution, as well as a stable fixation were enrolled. Exclusion criteria of the study were any signs of relevant anterior segment diseases, geographic atrophy, macular neovascularization, diabetic retinopathy, glaucoma, and inflammatory retinal diseases, as well as previous vitreoretinal surgery or laser treatment. If both eyes met the inclusion criteria, both were included. In addition, patients and control participants with refractive errors of more than 3 diopters spherical equivalent were excluded. All participants underwent a complete ophthalmologic examination including Early Treatment Diabetic Retinopathy Study visual acuity, LLVA testing, slit-lamp examination, and fundus biomicroscopy.

The study protocol was approved by the local ethics committee (Ethik-Kommission, Medizinische Fakultät, Rheinische Friedrich-Wilhelms-Universität; Lfd. no. 125/14) and complied with the tenets of the Declaration of Helsinki. After explanation of the study's nature and possible consequences of participation of this study, informed written consent was obtained from all participants of this study.

Retinal Imaging Protocol

Retinal imaging was performed according to a standardized retinal imaging protocol in patients and control participants after pupil dilatation with 1.0% tropicamide and 2.5% phenylephrine. High-speed combined and simultaneous confocal scanning laser ophthalmoscopy plus spectral-domain OCT imaging was performed using the Spectralis HRA+OCT (digital image resolution, 768 × 768 pixels; Heidelberg Engineering, Heidelberg, Germany) device. The imaging protocol further included color fundus photography, fundus autofluorescence (excitation, λ = 488 nm; emission, λ = 500−800 nm; minimum 15 frames), single horizontal and vertical combined confocal scanning laser ophthalmoscopy plus spectral-domain OCT scan through the fovea (30°; automatic real-time mode minimum 9 scans), and raster spectral-domain OCT scan (30° × 25°; automatic real-time mode minimum 9 frames; 61 B-scans; distance, 120 μm).

Fundus-Controlled Perimetry

As recently described in detail,[21,26] mesopic (Goldmann size III; 200 ms; 4-2 strategy; background luminance, 1.27 cd/m^2; 3° radius; and 1-pixel fixation ring) and scotopic (Goldmann size V; 200 ms; 4-2 strategy; background luminance, 0.0032 cd/m^2: 3° radius; and 1-pixel fixation ring) FCP of the retina with a 56-stimuli point test grid (10° × 10°) centered on the fovea was performed in patients and control participants using the Nidek MP-1S device (Nidek Technologies, Padova, Italy). For scotopic FCP testing, participants were dark adapted for 30 minutes before testing. Moreover, scotopic testing always began with a filter selection examination to overcome the limited dynamic range of

threshold values and determine the most appropriate neutral density filter (0.0 log unit, 1.0 log unit, 2.0 log unit) at each examination. Because of the difficulties of a direct comparison of test results between different filter groups, participants were enrolled only for the longitudinal analysis of this study if no change of the neutral density filter was conducted between follow-up visits.

Analysis of Retinal Thickness

For the analysis of retinal layer thickness, automated segmentation of the different retinal layers was performed by the manufacture's software (Spectralis Viewing Module 6.3.2.0; Heidelberg Engineering). The segmentation of the different retinal layers in each of the 61 B-scans was reviewed carefully and corrected manually if indicated. According to previous publications,[21,27,28] retinal layers were defined as follows. We defined the thickness from the internal limiting membrane to Bruch's membrane as the full retinal thickness (FRET). All layers between the internal limiting membrane and outer plexiform layer were defined as the inner retinal thickness (IRET). The retinal thickness of the outer nuclear layer (ONL) ranged from the outer plexiform layer to the external limiting membrane. Analogous to the study by Sadigh et al,[29] the Henle fiber layer was counted toward the ONL. The photoreceptor inner segment and outer segment (IS+OS) thickness was determined from the external limiting membrane to the putative interdigitation zone. The RPEDC ranged from the Bruch's membrane to the apex of the RPE layer, including all drusen material.

Pointwise Topographic Correlation of Retinal Sensitivity to Retinal Layer Thickness

As previously reported in detail,[30] for the analysis of retinal layer thickness at the corresponding stimulus location of FCP testing, thickness maps for each layer were transferred as tab-delimited files to Image J (National Institutes of Health, Bethesda, MD). The FCP data were registered to a spectral-domain OCT en face image using nonlinear affine transformation based on retinal vessel bifurcations. Then, corresponding thickness data at the precise site of the stimuli for mesopic (Goldmann III; retinal area, 0.43°) and scotopic (Goldmann V; retinal area, 1.7°) testing were generated for each layer using the measure function in Image J.

Statistical Analysis

Statistical analyses were performed using the analytics software environment R (R Foundation for Statistical Computing, Vienna, Austria). For the structure–function correlation, spectral-domain OCT data were standardized (Z scores) to fully account for topographic dependence of mean values and interindividual variability. The FCP data were normalized in terms of the sensitivity deviation from the normative mean of control participants (i.e., negative numbers denote sensitivity loss, positive numbers denote supranormal sensitivity). Mixed-effect models that included a random patient- and eye-specific intercept (to account factors such as lens opacification) were applied to analyze the effects of retinal layer thickness change and follow-up time on mesopic and scotopic retinal sensitivity. First, univariate linear mixed-effect models were fit to the data considering follow-up time as well as the individual layer thicknesses individually. Second, a multivariate model was fit to the data that included all layer thicknesses and the follow-up time.

Results

Demographics

Fifty-nine eyes of 54 AMD patients (mean age ± standard deviation [SD], 71.72 ± 8.97 years; interquartile range, 67.6–78.6 years; range, 45–85 years; 27 men; 19 pseudophakic eyes) and 27 eyes of 27 control participants (mean age ± SD, 64.65 ± 8.86 years; interquartile range, 57.7–72.0 years; range, 50–81 years; 22 men; 4 pseudophakic eyes) were included. The median best-corrected visual acuity was 0.1 logarithm of the minimum angle of resolution (Snellen equivalent, 20/25) for patients and 0.0 logarithm of the minimum angle of resolution (Snellen equivalent, 20/20) for control participants. In addition, for each patient and control participant, the LLVA vision and LLVA deficit were determined (Table 1). The median number of follow-up visits was 1 (range, 0–2), with a median follow-up time of 1.03 years (range, 0–2.07 years). Detailed results of patients and control demographics are shown in Tables 1 and 2.

Retinal Thickness Analysis

Regarding baseline results of the retinal thickness analysis in intermediate AMD patients compared with control participants, retinal thickness of the ONL was thinner by −0.49 SD (95% confidence interval [CI], −0.70 to −0.28 SD), whereas retinal thickness of the RPEDC layer was greater by 3.22 SD (95% CI, 2.27–4.17), respectively ($P < 0.001$). This corresponds in terms of thickness deviation (i.e., accounting for the spatial variation of normative mean thickness, but not for the spatial variation in normative thickness variability) to −4.94 µm for the ONL and +7.93 µm for the RPEDC. No statistically significant thickness changes at baseline were observed for the FRET, IRET, and PHOTORECEPTOR IS+OS retinal layers.

Longitudinally, we observed localized thickening of the RPEDC by +0.51 SD/year (95% CI, 0.28–0.74 SD/year; 1.23 µm/year), as well as a localized thinning of the ONL and PHOTORECEPTOR IS+OS by −0.03 SD/year (95% CI, −0.07 to 0.0 SD/year; −0.32 µm/year; $P = 0.045$) and −0.34 SD/year (95% CI, −0.40 to −0.27 SD/year; −0.76 µm/year; $P < 0.001$), respectively. For the FRET and IRET, follow-up time exhibited no effect on the thickness. Detailed results on the longitudinal thickness changes are shown in Table 3.

Functional Analysis

Compared with control participants, the mean pointwise sensitivity deviation at baseline in eyes with large drusen was reduced by an estimate of −1.67 dB (95% CI, −2.22 to −1.12 dB) for mesopic testing and by −2.34 dB (95% CI, −2.85 to −1.84 dB) for scotopic testing, respectively ($P < 0.001$). Per year of follow-up, pointwise sensitivity deviation in patients further decreased by −0.35 dB/year (95% CI, −0.43 to −0.28 dB/year) under mesopic and increased by +0.20 dB/year (95% CI, 0.12–0.29 dB/year) under scotopic testing conditions, respectively ($P < 0.001$). Results of the functional analysis are summarized in Table 4. A representative patient example with localized mesopic and scotopic retinal sensitivity change over time is illustrated in Figure 1.

Table 1. Demographic Baseline Characteristics at Baseline for Patients and Control Participants

	Patients (n = 59 Eyes)	Control Participants (n = 27 Eyes)
Age (yrs), mean ± SD*	71.72 ± 8.97	64.65 ± 8.86
Visual acuity (logMAR)		
BCVA	0.1	0.0
LLVA	0.4	0.3
LLVA deficit	0.3	0.3
Lens status		
Phakic	40	23
Pseudophakic	19	4

BCVA = best-corrected visual acuity; LLVA = low-luminance visual acuity; logMAR = logarithm of the minimum angle of resolution; SD = standard deviation.
*All patients and control participants.

Structure–Function Correlation

For structure–function correlation in patients, univariate linear mixed-effect models were applied to separately consider the individual impact of single retinal layer thickness changes or the impact of follow-up time alone on pointwise mesopic and scotopic retinal sensitivity loss. In detail, retinal thickness of the IRET and the PHOTORECEPTOR IS+OS retinal layers showed no significant influence on pointwise mesopic and scotopic sensitivity loss, whereas a significant correlation was exhibited for the FRET, the ONL, and the RPEDC thickness for both types of sensitivity testing, respectively ($P < 0.001$).

In terms of pointwise sensitivity change for each normative SD of retinal layer thickening, results of the univariate linear mixed-effect models exhibited the most pronounced association of mesopic sensitivity with the ONL (+0.25 dB/SD_{ONL}; 95% CI, 0.20–0.30 dB/SD_{ONL}), followed by the FRET (−0.18 dB/SD_{FRET}; 95% CI, −0.24 to −0.11 dB/SD_{FRET}) and the RPEDC thickness (−0.05 dB/SD_{RPEDC}; 95% CI, −0.05 to −0.04 dB/SD_{RPEDC}), respectively ($P < 0.001$). Within this model, the association with pointwise scotopic retinal sensitivity was even higher for the ONL (+0.48 dB/SD_{ONL}; 95% CI, 0.41–0.54 dB/SD_{ONL}), followed by the FRET (−0.20 dB/SD_{FRET}; 95% CI, −0.27 to −0.13 dB/SD_{FRET}) and the RPEDC thickness (−0.05 dB/SD_{RPEDC}; 95% CI, −0.06 to −0.05 dB/SD_{RPEDC}), respectively ($P < 0.001$). Moreover, during the 3-year follow-up time, a significant association with pointwise mesopic and scotopic sensitivity loss, respectively, was found ($P < 0.001$). Detailed results of the univariate linear mixed-effect models are demonstrated in Supplemental Table S1 (available at www.ophthalmologyretina.org).

Regarding results from the combined linear mixed-effect models in patients taking all retinal layer thicknesses as well as follow-up time together into consideration to account for the predominant parameter on retinal function, retinal thickness of the ONL ($P < 0.001$) and the RPEDC ($P = 0.014$) layer exhibited a significant effect on mesopic sensitivity loss, whereas for scotopic testing, a significant association was shown for the FRET ($P < 0.001$), IRET ($P = 0.002$), ONL ($P < 0.001$), and PHOTORECEPTOR IS+OS ($P < 0.001$) thickness. Moreover, follow-up time remained a significant predictor of mesopic and scotopic retinal function, respectively ($P < 0.001$). A graphical illustration of the dependence of retinal layer thickness and follow-up time on mesopic and scotopic retinal sensitivity is provided in Figure 2. A summarizing table of the results of the multivariate combined linear mixed-effect models in patients is provided in Supplemental Table S2 (available at www.ophthalmologyretina.org).

Exclusion of Sensitivity Values Close to Floor and Ceiling Effects

After exclusion of all sensitivity test point values of less than 2 dB and more than 18 dB because of potential floor and ceiling effects (related to the limited dynamic range of the device), mean pointwise sensitivity loss estimate for baseline changed considerably for mesopic testing (−2.46 dB ; 95% CI, −2.89 to −2.04 dB) and slightly for scotopic testing (−2.56 dB; 95% CI, −2.96 to −2.16 dB). Estimates for change in sensitivity over time remained similar, with rates of −0.28 dB/year (95% CI, −0.36 to −0.20 dB/year) for mesopic testing and +0.13 dB/year (95% CI, 0.05–0.21 dB/year)

Table 2. Overview of the Number of Follow-up Visits and Reasons for Study Exit in the Patient Cohort

	Baseline	Follow-up 1	Follow-up 2	Follow-up 3
Total no. of assessed patients' eyes for analysis	59	38	25	14
No. of study exits				
Conversion to late-stage AMD*	—	4	2	1
ND filter change	—	4	2	4
General illness	—	4	0	0
Withdrawn consent	—	9	9	6

AMD = age-related macular degeneration; ND = neutral density; — = not applicable.
*Included presence of complete retinal pigment epithelium and outer retinal atrophy.[25]

Table 3. Univariate Linear Mixed-Effects Model Estimates for the Change in Retinal Layer Thicknesses over Time (Outcome Unit, Z Score [Number of Normative Standard Deviations from the Normative Mean]), 95% Confidence Interval, and Corresponding *P* Values for Retinal Thickness Changes in Patients at Baseline (Intercept) and over Time

Parameter	Estimates (as Z Score)	95% Confidence Interval	*P* Value	Estimates (as Thickness Deviation)
FRET thickness				
Baseline (intercept)	0.07 SD	−0.14 to 0.27	0.519	1.83 µm
Follow-up time	0.01 SD/yr	−0.02 to 0.03	0.565	0.21 µm/yr
IRET thickness				
Baseline (intercept)	−0.06 SD	−0.23 to 0.12	0.525	−1.43 µm
Follow-up time	0.00 SD/yr	-0.02 to 0.02	0.974	0.01 µm/yr
ONL thickness				
Baseline (intercept)	−0.49 SD	−0.70 to −0.28	**< 0.001**	−4.94 µm
Follow-up time	−0.03 SD/yr	−0.07 to 0.00	**0.045**	−0.32 µm/yr
Photoreceptor IS+OS thickness				
Baseline (intercept)	0.10 SD	−0.14 to 0.33	0.417	0.21 µm
Follow-up time	−0.34 SD/yr	−0.40 to −0.27	**< 0.001**	−0.76 µm/yr
RPEDC thickness				
Baseline (intercept)	3.22 SD	2.27–4.17	**< 0.001**	7.93 µm
Follow-up time	0.51 SD/yr	0.28–0.74	**< 0.001**	1.23 µm/yr

FRET = full retinal thickness; IRET = inner retinal thickness; IS+OS = inner segment and outer segment; ONL = outer nuclear layer; RPEDC = retinal pigment epithelium–drusen complex; SD = standard deviation.
For comparison, we also provide the estimates in terms of thickness deviation from the spatially corresponding normative value. However, in contrast to the Z scores, these do not take into account that the normative variability in thickness varies across the retina. Boldface values indicate statistical significance at $P < 0.05$.

for scotopic testing ($P < 0.001$). Likewise, the structure–function analyses yielded similar results.

Discussion

This longitudinal structure–function analysis in eyes with large drusen demonstrated progressive thinning of outer retinal layers that correlated with a localized loss of mesopic retinal sensitivity and even more pronounced loss of scotopic retinal sensitivity in eyes with intermediate AMD. Furthermore, we showed an independent effect of follow-up time on retinal sensitivity.

Regarding results from the functional analysis, our findings of a stronger impairment of scotopic than mesopic retinal function within intermediate AMD eyes are in line with previous published findings.[26,31,32] In detail in our study, mean baseline retinal sensitivity was reduced by −1.67 dB for mesopic testing and −2.34 dB for scotopic testing. Moreover, the result of the longitudinal mesopic sensitivity loss of −0.35 dB/year in intermediate AMD eyes is comparable with previous findings by Wu et al[33,34] reporting a mean pointwise sensitivity change over 12 months of −0.42 dB and also with recent findings on mesopic FCP testing in the nonstudy eyes of the LEAD Study. In another report by Hsu et al[35] an average mesopic threshold change of −3.0 dB within 12 months of follow-up time was demonstrated. Despite of the use of different FCP devices and test patterns, all of these results point toward measurable changes in eyes with intermediate AMD within 1 year.

Although results on mesopic testing are plausible and comparable between recently published studies, current results on longitudinal scotopic retinal function remain ambiguous. Corresponding to our findings at baseline, that is, a stronger reduction of scotopic than mesopic retinal function, we expected to observe a more pronounced decline of scotopic retinal function within the longitudinal period.

Table 4. Results of the Pointwise Sensitivity Loss (Outcome Unit: Sensitivity Deviation from the Normative Mean in Decibels [Negative Numbers Indicate Loss of Function]), the 95% Confidence Interval, and the Corresponding *P* Values for Retinal Mesopic and Scotopic Sensitivity Tested by Fundus-Controlled Perimetry in Patients at Baseline (Intercept) and over Time

Parameter	Pointwise Sensitivity Loss (dB)	95% Confidence Interval	*P* Value
Mesopic FCP			
Baseline (intercept)	−1.67	−2.22 to −1.12	**< 0.001**
Change/yr	−0.35	−0.43 to −0.28	**< 0.001**
Scotopic FCP			
Baseline (intercept)	−2.34	−2.85 to −1.84	**< 0.001**
Change/yr	+0.20	+0.12 to +0.29	**< 0.001**

FCP = fundus-controlled perimetry.
Boldface values indicate statistical significance at $P < 0.05$.

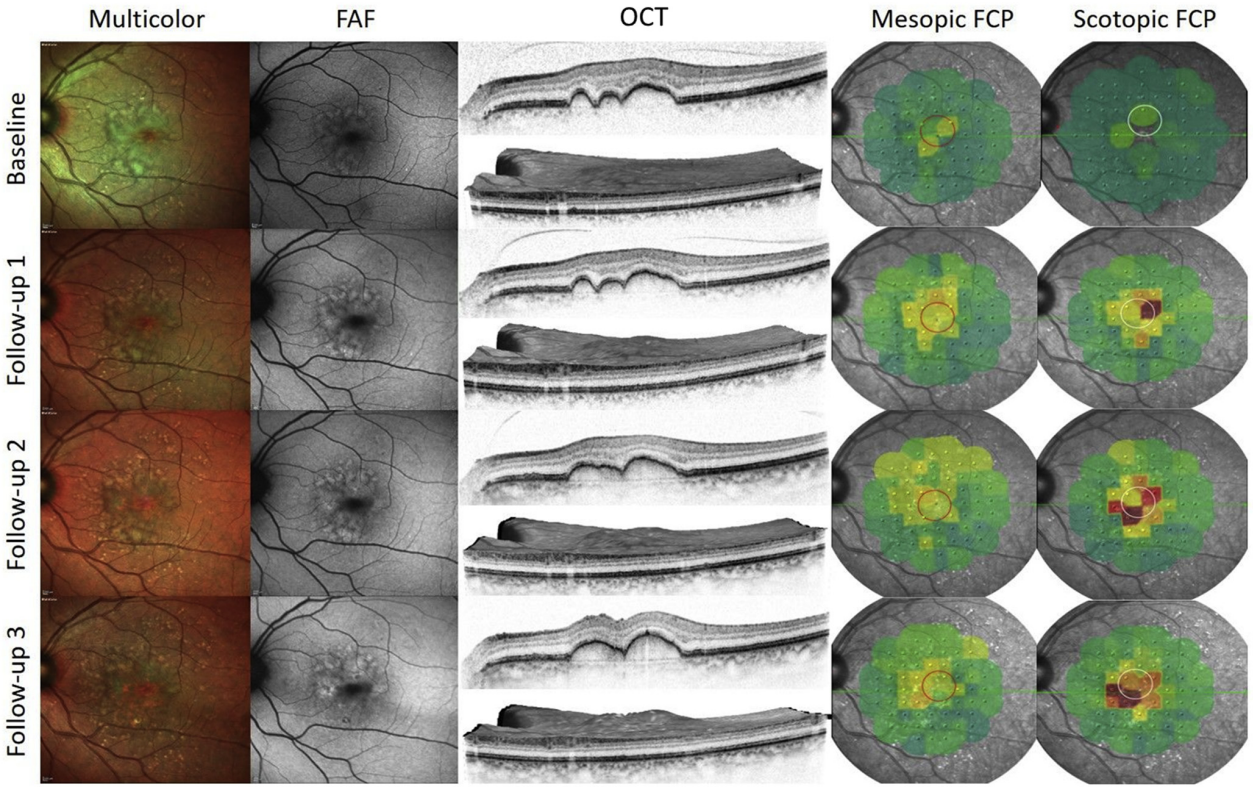

Figure 1. Representative patient example showing large drusen secondary to intermediate age-related macular degeneration within 4 examination visits. Multimodal imaging with multicolor, fundus autofluorescence (FAF), and OCT B-scan imaging was applied at all visits. Note the enlargement of central large drusen corresponding to a more pronounced central decline of scotopic, rather than mesopic, retinal sensitivity on fundus-controlled perimetry (FCP) examination. Progressive decline of retinal sensitivity is highlighted by orange and red fields in FCP testing images.

However, this study showed a trend toward a slight improvement of scotopic sensitivity values in patients. Although those results seem ambiguous, one has to note that patients' visits were excluded from further analysis in case of a change in the neutral density filter during follow-up visits. Because a predominant change to a weaker neutral density filter was made in those patients during follow-up visits, we assume an overall higher loss of scotopic retinal sensitivity among excluded patients within the longitudinal period. Accordingly, the herein observed significant change over time constitutes a rather conservative estimate.

Because scotopic retinal sensitivity is affected more highly at baseline than mesopic retinal function, our baseline results are in line with previous reports of a supposed higher vulnerability of rod than cone photoreceptors within AMD.[17,36,37] Previous reported reasons are differences in the retinoid delivery pathways leading to a lack of vitamin A, which is associated primarily with rod rather than cone degeneration. Moreover presence, of genetic variations, for example *CFH* and *ARMS2* polymorphisms, have been associated with a significantly higher rod intercept time.[37,38]

Thus, it may be possible that within a defined follow-up time of 3 years, mesopic impairment is succeeding previously reduced scotopic function, whereas scotopic function remains stable or rather varies slightly within patient individual testing within a 3-year follow-up period. In this context, it is imaginable that further scotopic impairment could be observed over a longer follow-up period. Future long-term follow-up examinations and bigger sample sizes are needed for further validation.

Regarding the results from the structural analysis, baseline findings of localized ONL thinning as well as localized RPEDC thickening in the presence of drusen has already been demonstrated in prior studies.[12,39,40] In this study, we further observed a significant longitudinal RPEDC thickening (+0.51 SD/year), as well as ONL and PHOTORECEPTOR IS+OS thinning (−0.03 SD/year and −0.34 SD/year, respectively). Although further detailed observational studies on retinal layer thickness change are currently not available, interestingly, results of this analysis seem to suggest similar trends as previous findings by Lamin et al,[41] who demonstrated an increasing RPE−Bruch's membrane volume by 0.0209 SD ($P = 0.000$) and a decreasing ONL volume by 0.0851 SD ($P = 0.001$) from baseline to year 2 in AMD eyes compared with healthy control participants. Although no significant reduction of the photoreceptor volume (IS+OS) at baseline was found in the study by Lamin et al, several examples of loss of ISOS layer thickness above drusen have been reported in cross-sectional studies.[12,29,41] A progressive thinning of the PHOTORECEPTOR IS+OS

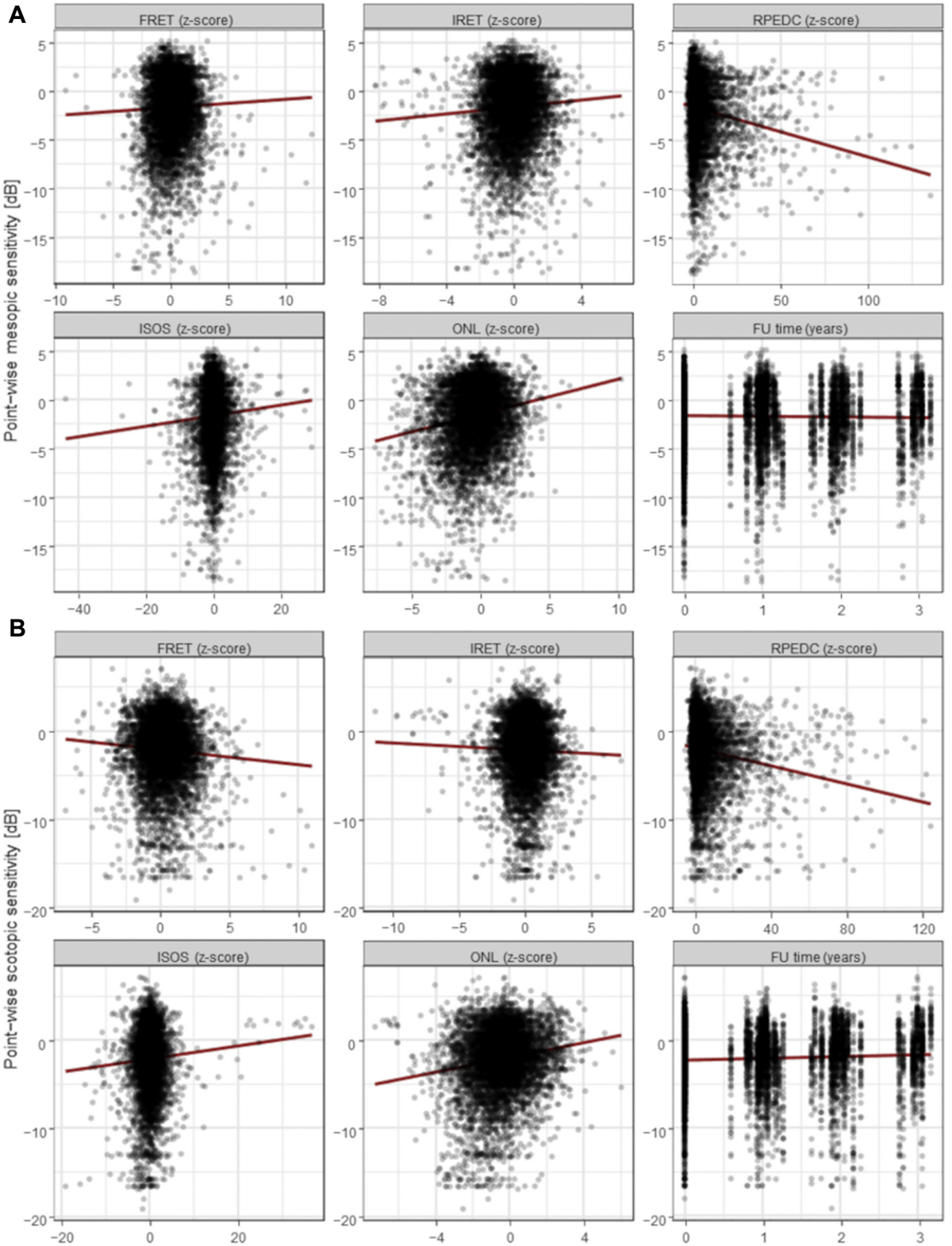

Figure 2. Scatterplots showing the pointwise (**A**) mesopic and (**B**) scotopic retinal sensitivity (in decibels) in dependence of retinal thickness for full retinal thickness (FRET), inner retinal thickness (IRET), retinal pigment epithelium–drusen complex (RPEDC), photoreceptor inner segment and outer segment (IS+OS), outer nuclear layer (ONL), and dependence of follow-up (FU) time. Thickness data were plotted in terms of standard deviation of the normative mean (Z scores). Follow-up time was plotted in years.

layer within the longitudinal period by −0.34 SD/year (corresponding −0.76 μm/year) were shown in this study.

Regarding the structure−function correlation, this study underlined recent findings by Wu et al,[24] who demonstrated a significant correlation of localized RPEDC thickening and localized presence of hyperreflective foci to reduced mesopic retinal sensitivity in FCP testing over 12 months. Interestingly, the most significant associations of retinal layer thickness with both mesopic and scotopic function were demonstrated in this work for the FRET, ONL, and RPEDC layer. However, taking pointwise sensitivity losses for those layers into consideration, a trend was found toward higher sensitivity losses for scotopic testing at baseline. These findings can strengthen previous assumptions of a higher vulnerability of rods than cones within AMD disease. Moreover, these findings on the association of structural to functional changes show that both of the types of measure may serve as a clinical outcome in future interventional AMD trials.

Moreover, despite taking the FRET, the ONL, and the RPEDC layer into account, follow-up time still showed a significant correlation with mesopic and scotopic retinal sensitivity, in contrast to a previous published work by Wu et al[24] reporting on the correlation of microstructural changes with mesopic FCP testing within a period of 12 months. The precise reason for this observation is unclear at this point. Because retinal layer thickness measurements may be insufficient in predicting retinal sensitivity alone, more complex models taking additional parameters, as for example, presence of hyperreflective foci as well as ellipsoid zone integrity, into consideration are further needed to uncover nonlinear associations between retinal layer thicknesses and retinal sensitivity as well as interaction effects that may be more predictive of retinal function. Artificial intelligence-based statistical analysis models, for example, as performed in a work by von der Emde et al[42] and Pfau et al,[43] enable estimation of various effects such as follow-up time and also various abnormal retinal structural changes on mesopic and scotopic retinal function that can potentially serve as functional surrogate end points in future AMD trials.

Moreover, for the design of future clinical trials, one important question is related to the observation time that would be needed for detection of progressive disease-related changes by imaging and functional testing. In this context, one important result of the current study is the finding that progressive disease-related thickness changes of the ONL and the RPDEC layers were observable within 1 year and paralleled by a longitudinal decline of mesopic sensitivity.

Several limitations of this study need to be considered. The number of patients undergoing the extensive structural and functional tests in this study was limited and the number of follow-up visits was inhomogeneous, ranging from 0 to 3. At the third follow-up, we observed that 24 patients had withdrawn consent and exited the study. We believe that this high number reflects the exhausting and time-consuming examination of FCP, including dark adaptation, in the elderly patients affected by AMD. For control participants, only data at baseline were available. Because we are not able to provide longitudinal data for control participants of this age group by using the same equipment, it cannot be excluded that both the thickness changes in retinal layers and decrease in retinal sensitivity that were observed in patients were driven solely by the disease or additionally by the normal effects of aging. A major limitation remains the limited dynamic range of the FCP device used in this study. It is reassuring that the functional results and correlation to structure were similar when excluding retinal sensitivity values at both the upper and lower range.[44] Using sensitivity loss as dependent variable, we accounted for the spatial variation of the average normative sensitivity. However, *Z* score transformation (to adjust further for spatial differences in between-patient variability in normative sensitivity) was not possible because of the limited dynamic range of the device, resulting in skew of the normal data.[44]

In conclusion, we demonstrated that AMD-related thickness changes of outer retinal layers could be quantified in the intermediate stage of the disease and are quantifiable within a relatively short period of 1 year. Further, progressive functional impairment that was mostly spatially confined to localized thickness changes was detectable. Both mesopic and scotopic sensitivity losses were observed, although the latter exceeded the former. Because longitudinal sensitivity changes could not be explained by retinal thickness changes alone in this study, we conclude that more complex models including additional refined parameters on retinal structure and possibly other factors are still needed to improve prediction of retinal function.

These findings are in accordance with the current assumptions regarding progressive photoreceptor degeneration that occurs in AMD, affecting rod function to a larger degree compared with cone function. These findings may add to a better understanding of dynamic structural changes and functional impairment in eyes with intermediate AMD.

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References

1. Lim LS, Mitchell P, Seddon JM, et al. Age-related macular degeneration. *Lancet*. 2012;379:1728−1738.
2. Holz FG, Tadayoni R, Beatty S, et al. Multi-country real-life experience of anti-vascular endothelial growth factor therapy for wet age-related macular degeneration. *Br J Ophthalmol*. 2015;99:220−226.
3. Volz C, Pauly D. Antibody therapies and their challenges in the treatment of age-related macular degeneration. *Eur J Pharm Biopharm*. 2015;95:158−172.
4. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of age-related macular degeneration in Europe: the past and the future. *Ophthalmology*. 2017;124:1753−1763.
5. Ferris FL, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology*. 2013;120:844−851.
6. Auge J, Steinberg JS, Fleckenstein M, et al. Reticular drusen over time with SD-OCT. *Ophthalmologe*. 2014;111:765−771.
7. Thiele S, Nadal J, Fleckenstein M, et al. Longitudinal analysis of drusen volume in intermediate age-related macular degeneration using two spectral-domain optical coherence

tomography scan patterns. *Ophthalmologica*. 2018;239:110–120.
8. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular degeneration: AREDS report no. 18. *Arch Ophthalmol*. 2005;123:1570–1574.
9. Thiele S, Nadal J, Pfau M, et al. Prognostic value of retinal layers in comparison with other risk factors for conversion of intermediate age-related macular degeneration. *Ophthalmol Retina*. 2019;4:31–40.
10. Schmitz-Valckenberg S, Steinberg JS, Fleckenstein M, et al. Combined confocal scanning laser ophthalmoscopy and spectral-domain optical coherence tomography imaging of reticular drusen associated with age-related macular degeneration. *Ophthalmology*. 2010;117:1169–1176.
11. Rogala J, Zangerl B, Assaad N, et al. In vivo quantification of retinal changes associated with drusen in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:1689–1700.
12. Schuman SG, Koreishi AF, Farsiu S, et al. Photoreceptor layer thinning over drusen in eyes with age-related macular degeneration imaged in vivo with spectral-domain optical coherence tomography. *Ophthalmology*. 2009;116:488–496.e2.
13. Cocce KJ, Stinnett SS, Luhmann UFO, et al. Visual function metrics in early and intermediate dry age-related macular degeneration for use as clinical trial endpoints. *Am J Ophthalmol*. 2018;189:127–138.
14. Dimitrov PN, Robman LD, Varsamidis M, et al. Visual function tests as potential biomarkers in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:9457–9469.
15. Pondorfer SG, Terheyden JH, Heinemann M, et al. Association of vision-related quality of life with visual function in age-related macular degeneration. *Sci Rep*. 2019;9:15326.
16. McGuinness MB, Finger RP, Wu Z, et al. Properties of the impact of vision impairment and night vision questionnaires among people with intermediate age-related macular degeneration. *Transl Vis Sci Technol*. 2019;8:3.
17. Owsley C, McGwin G, Jackson GR, et al. Cone- and rod-mediated dark adaptation impairment in age-related maculopathy. *Ophthalmology*. 2007;114:1728–1735.
18. Finger RP, Schmitz-Valckenberg S, Schmid M, et al. MACUSTAR: development and clinical validation of functional, structural, and patient-reported endpoints in intermediate age-related macular degeneration. *Ophthalmologica*. 2019;241:61–72.
19. Wu Z, Ayton LN, Guymer RH, Luu CD. Low-luminance visual acuity and microperimetry in age-related macular degeneration. *Ophthalmology*. 2014;121:1612–1619.
20. Cassels NK, Wild JM, Margrain TH, et al. The use of microperimetry in assessing visual function in age-related macular degeneration. *Surv Ophthalmol*. 2018;63:40–55.
21. Saßmannshausen M, Steinberg JS, Fimmers R, et al. Structure-function analysis in patients with intermediate age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2018;59:1599–1608.
22. Vujosevic S, Pucci P, Casciano M, et al. Long-term longitudinal modifications in mesopic microperimetry in early and intermediate age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2017;255:301–309.
23. Nguyen CT, Fraser RG, Tan R, et al. Longitudinal changes in retinotopic rod function in intermediate age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2018;59.AMD19–AMD24.
24. Wu Z, Cunefare D, Chiu E, et al. Longitudinal associations between microstructural changes and microperimetry in the early stages of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2016;57:3714–3722.
25. Sadda SR, Guymer R, Holz FG, et al. Consensus definition for atrophy associated with age-related macular degeneration on OCT: Classification of Atrophy report 3. *Ophthalmology*. 2018;125:537–548.
26. Steinberg JS, Fitzke FW, Fimmers R, et al. Scotopic and photopic microperimetry in patients with reticular drusen and age-related macular degeneration. *JAMA Ophthalmol*. 2015;133:690–697.
27. Yoshimura N, Hangai M. *OCT Atlas*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014.
28. Greferath U, Guymer RH, Vessey KA, et al. Correlation of histologic features with in vivo imaging of reticular pseudodrusen. *Ophthalmology*. 2016;123:1320–1331.
29. Sadigh S, Cideciyan AV, Sumaroka A, et al. Abnormal thickening as well as thinning of the photoreceptor layer in intermediate age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2013;54:1603–1612.
30. Pfau M, Lindner M, Gliem M, et al. Mesopic and dark-adapted two-color fundus-controlled perimetry in patients with cuticular, reticular, and soft drusen. *Eye (Lond)*. 2018;32:1819–1830.
31. Pfau M, Lindner M, Fleckenstein M, et al. Test-retest reliability of scotopic and mesopic fundus-controlled perimetry using a modified MAIA (Macular Integrity Assessment) in normal eyes. *Ophthalmologica*. 2017;237:42–54.
32. Nebbioso M, Barbato A, Pescosolido N. Scotopic microperimetry in the early diagnosis of age-related macular degeneration: preliminary study. *Biomed Res Int*. 2014;2014:671529.
33. Wu Z, Ayton LN, Luu CD, Guymer RH. Longitudinal changes in microperimetry and low luminance visual acuity in age-related macular degeneration. *JAMA Ophthalmol*. 2015;133:442–448.
34. Wu Z, Luu CD, Hodgson LAB, et al. Secondary and exploratory outcomes of the subthreshold nanosecond laser intervention randomized trial in age-related macular degeneration: a LEAD Study report. *Ophthalmol Retina*. 2019;3:1026–1034.
35. Hsu ST, Thompson AC, Stinnett SS, et al. Longitudinal study of visual function in dry age-related macular degeneration at 12 months. *Ophthalmol Retina*. 2019;3:637–648.
36. Steinberg JS, Saßmannshausen M, Fleckenstein M, et al. Correlation of partial outer retinal thickness with scotopic and mesopic fundus-controlled perimetry in patients with reticular drusen. *Am J Ophthalmol*. 2016;168:52–61.
37. Curcio CA, Owsley C, Jackson GR. Spare the rods, save the cones in aging and age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2000;41:2015–2018.
38. Mullins RF, McGwin G, Searcey K, et al. The ARMS2 A69S polymorphism is associated with delayed rod-mediated dark adaptation in eyes at risk for incident age-related macular degeneration. *Ophthalmology*. 2019;126:591–600.
39. Pappuru RR, Ouyang Y, Nittala MG, et al. Relationship between outer retinal thickness substructures and visual acuity in eyes with dry age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:6743–6748.
40. Wu Z, Ayton LN, Luu CD, Guymer RH. Relationship between retinal microstructures on optical coherence tomography and microperimetry in age-related macular degeneration. *Ophthalmology*. 2014;121:1445–1452.
41. Lamin A, Oakley JD, Dubis AM, et al. Changes in volume of various retinal layers over time in early and intermediate age-related macular degeneration. *Eye (Lond)*. 2019;33:428–434.

42. von der Emde L, Pfau M, Dysli C, et al. Artificial intelligence for morphology-based function prediction in neovascular age-related macular degeneration. *Sci Rep*. 2019;9:11132.
43. Pfau M, von der Emde L, Dysli C, et al. Determinants of cone- and rod-function in geographic atrophy: AI-based structure-function correlation. *Am J Ophthalmol*. 2020;In press.
44. Bowl W, Lorenz B, Jäger M, Friedburg C. Improving detection of mild loss of retinal light increment sensitivity at the posterior pole with the microperimeter MP1. *Invest Ophthalmol Vis Sci*. 2013;54:4666–4674.

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No animal subjects were included in this study.

Author Contributions:

Conception and design: Saßmannshausen, Zhou, Pfau, Thiele, Steinberg, Fleckenstein, Holz, Schmitz-Valckenberg

Analysis and interpretation: Saßmannshausen, Zhou, Pfau, Thiele, Steinberg, Fleckenstein, Holz, Schmitz-Valckenberg

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Obtained funding: Pfau, Thiele, Fleckenstein

Overall responsibility: Saßmannshausen, Zhou, Pfau, Thiele, Steinberg, Fleckenstein, Holz, Schmitz-Valckenberg

Abbreviations and Acronyms:
CI = confidence interval; **FCP** = fundus-controlled perimetry; **FRET** = full retinal thickness; **IRET** = inner retinal thickness; **IS+OS** = inner segment and outer segment; **LLVA** = low-luminance visual acuity; **ONL** = outer nuclear layer; **RPE** = retinal pigment epithelium; **RPEDC** = retinal pigment epithelium–drusen complex; **SD** = standard deviation.

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