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Retina

Photostress Recovery Time as a Potential Predictive Biomarker for Age-Related Macular Degeneration

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Purpose: The purpose of this study was to assess recovery time following photostress and its association with age-related macular degeneration (AMD) cross-sectionally and longitudinally in an elderly population-based cohort.

Methods: We analyzed photostress recovery time (PRT) and AMD in >1800 AugUR study participants aged 70+ years. On color fundus images from baseline and 3-year follow-up, presence of AMD was graded manually (Three Continent AMD Consortium Severity Scale). Visual acuity (VA) was assessed via Early Treatment Diabetic Retinopathy Study (ETDRS) charts. After a 30-second bleaching of the macular region via direct ophthalmoscope, PRT was measured as the seconds to regain VA.

Results: First, we analyzed 1208 AugUR participants cross-sectionally (288 with early AMD, and 78 with late AMD). Prolonged PRT was associated with early and late AMD versus no AMD (median PRT = 119.5, 198.0 versus 80.0 seconds, respectively; logistic regression odds ratio [OR] = 1.109–1.165 per 10 seconds, P values < 0.0001). Sensitivity analyses using alternative models or restricting to participants after cataract surgery revealed similar ORs. Second, the association was confirmed in an independent cross-sectional AugUR sample (n = 486). Third, in longitudinal analysis of 233 AugUR participants without AMD, prolonged PRT was associated with incident AMD ascertained 3 years later (follow-up time = 3.2 ± 0.2 years, OR = 1.112–1.162 per 10 seconds, P < 0.05). Overall, we demonstrate a significant association of prolonged PRT with AMD cross-sectionally and longitudinally in elderly individuals.

Conclusions: Prolonged PRT might capture retinal function impairment after cell damage before early AMD is visible via color fundus imaging.

Translational Relevance: Our results suggest PRT as quantitative predictive biomarker for incident AMD, making it potentially worthwhile also for clinical care.

Introduction

Age-related macular degeneration (AMD) represents the leading cause of irreversible central vision impairment in the older populations of industrialized countries.[1] This degenerative disorder of the central retina is caused by disturbances in the functional syncytium of choroid, retinal pigment epithelium (RPE), and photoreceptors.[1] During the course of disease, cell damage in the RPE and photoreceptors becomes structurally visible as early and late disease stages. Early AMD is determined by differently sized yellowish accumulations of extracellular material (basal linear deposits/drusen in the sub-RPE basal lamina space and subretinal drusenoid deposits) or abnormalities of the RPE, including depigmentation or increased pigmentation.[1–3] Late AMD can appear as a neovascular (NV) complication or an atrophic form known as geographic atrophy (GA) of the RPE.[1] These structural features of early and late AMD can be detected and graded via color fundus photography, a widely used gold standard for epidemiological studies.[4–6]

A variety of tests are available to assess retinal function in AMD. Best-corrected visual acuity (VA) is often unaffected by early AMD, whereas late AMD is vision impairing. Therefore, VA is not a sensitive functional measure until the late stages of disease.[7] Other tests, for example, under low contrast and low luminance, better assess visual function in earlier AMD disease stages.[8]

Delayed rod-mediated dark adaption (RMDA) has recently been postulated as one of the first signs of visual dysfunction in early AMD and as a functional biomarker for incident early AMD.[9,10] Previous evidence also suggests that cone-mediated adaption after exposure to glare is commonly slowed down in AMD, even among patients with normal VA, and that it can vary with age and extent of disease.[11] Recovery of cone photoreceptor function after glare can be assessed with the photostress test as the photostress recovery time (PRT).[11–19] The photostress test is easy to perform: in principle, the eye is exposed to intense light, and then the time until the VA returns to a predetermined VA level is recorded. Measuring either RMDA or PRT assesses the efficiency of the visual cycle (in rods or cones, respectively). Both approaches begin with photopigment depletion following exposure to intense light and have a sensitivity recovery period. The duration of this period depends on the rate at which photopigment regenerates.[9,12]

It has been shown that PRT was prolonged in individuals with features of early or late AMD visible on color fundus images.[11,16] This is in line with a hypothesis that prolonged PRT is a marker of impaired visual cycle in patients with AMD. Furthermore, it is perceivable that retinal cell damage which is not yet structurally visible as AMD on color fundus images might already impair the visual cycle. We thus hypothesize that an impaired visual cycle can be captured by a prolonged PRT before structural AMD features are visible on color fundus images.

PRT has not yet been assessed in epidemiological studies on AMD. Both cross-sectional and longitudinal data from population-based cohorts are lacking. Cross-sectional evaluations are hampered by the fact that AMD is frequent rather at old age and that the old-aged are typically under-represented in population-based cohort studies. Old-aged study participants have special needs due to impaired mobility, hearing, or vision, thus requiring tailored study protocols. Longitudinal data on incident AMD is challenging as it either requires a very long follow-up of a general adult cohort or the recruiting of the old-aged with the above stated needs. General population-based studies often exclude the old-aged (e.g. NAKO, age of participants 20–69 years[20]; and UK Biobank, 40–69 years[21]). Previous studies on AMD including old-aged individuals (e.g. Beaver Dam, 43–86 years[22]; Japan Public Health Center–Based Prospective Study, 65–86 years[23,24]) have not integrated a photostress test.

We aimed to investigate the value of PRT as a concurrent or even predictive marker of AMD. For this, we implemented the photostress test in our population-based AugUR cohort study (Altersbezogene Untersuchungen zur Gesundheit der Universität Regensburg) in individuals aged 70+ years.[25] AugUR is designed to investigate chronic diseases typical at old age cross-sectionally as well as longitudinally, with a special focus on degenerative eye disorders. The target population of 70+ years had been chosen to enable the observation of incident AMD within a relatively short follow-up. In AugUR, we previously estimated early and late AMD prevalence[6] as well as incidence[26] using color fundus imaging. In this present analysis of a total of approximately 1800 individuals aged 70 to 98 years, we (i) investigated the association of PRT with AMD status cross-sectionally in two independent surveys, thus enabling replication. (ii) Given the strong genetic influence on AMD,[27] we evaluated association of the AMD genetic risk score (GRS)[26] with PRT to substantiate the PRT association with AMD – rather than with age. (iii) We investigated the potential of PRT to predict late as well as early AMD, by longitudinal analyses in an independent subset of AugUR participants with 3-year follow-up.

Subjects and Methods

Study Population, Study Sample, and Data Collection

AugUR is a population-based cohort study recruiting from the mobile elderly population in/around Regensburg, Germany, a study region with approximately 350,000 inhabitants of mostly Caucasian ancestry. AugUR recruitment was conducted in two independent baseline surveys (AugUR1, 2013–2015; and AugUR2 2017–2019). We here present results from both baseline surveys as well as the 3-year and 6-year follow-up of AugUR1. Study recruitment and conduct for both surveys, baseline, and follow-up, were similar and have been described previously.[6,25] Briefly, inhabitants of the city and county of Regensburg, Germany, with ≥70 years of age, were identified by local registries and invited by a mailed written invitation letter to the study center at the Regensburg University Hospital. Individuals were included into the AugUR study, if they were able and willing to come to the study center, to participate in a 3-hour study program, and to

provide informed written consent. There were no exclusion criteria. Information on lifestyle factors, metabolic parameters, general and ocular comorbidities, including ocular interventions, such as cataract surgery, were then gathered via a standardized face-to-face interview, medical examinations by trained medical staff, and laboratory measurements from blood or urine.[6,25]

The AugUR study was approved by the Ethics Committee of the University of Regensburg, Germany (vote 12-101-0258). The study complies with the Declaration of Helsinki and its later amendments. All participants provided informed written consent.

AMD Classification Based on Color Fundus Images

Color fundus photography of the central retina and assessment of AMD features were conducted as described previously[6,25,26]; they were the same for the baseline and follow-up assessments. For each eye, presence, size and area of drusen, pigment abnormalities (hyperpigmentation or depigmentation), GA, or NV was determined using gradable color fundus images. This information was then transferred into the AMD status per eye according to the Three Continent AMD Consortium Severity Scale (3CACSS) as no AMD, mild/moderate/severe early AMD, or late AMD. Finally, the AMD status of a person was derived as the AMD status of the eye with the more severe stage ("worse eye") when color fundus images of both eyes were gradable for AMD. When images were gradable only for one eye, the AMD status of the person was the AMD status of this eye. We analyzed individuals with at least one gradable eye.

Of note, the "mild/moderate/severe early AMD" categories by 3CACSS have been previously shown to be congruent with the "intermediate AMD" category by the Clinical Classification (CC; i.e. they distinguish different stages within the CC´s "intermediate AMD".)[6]

Measurement of Photostress Recovery Time

For measuring PRT, we chose a previously established test approach, which involves high intensity and long duration bleaching[12]: this protocol includes a long light exposure time of 30 seconds. It ensures that a sufficient amount of photopigment is bleached and thus reduces the effect of small timing errors, which might derive from involuntary blinking or loss of fixation by, for example, involuntary eye movements. This approach had been shown to be least prone to variability and closest to a standardized reference technique when compared to other methods (e.g. using shorter bleaching times and only a pen torch). The reference technique is a laboratory rather than clinical technique using a single channel Maxwellian view optical system, which thus requires expensive equipment and more time.[12]

The following measurements were conducted for each eye separately while covering the other eye: first, initial VA was evaluated and documented in logMAR via standard Early Treatment Diabetic Retinopathy Study (ETDRS) charts in 4 m distance. Participants were asked to wear their own glasses / correction if applicable. The best acuity (at least 60% of letters of the line read correctly) was marked on the chart.

Second, a direct ophthalmoscope (HEINE BETA 200; HEINE Optotechnik, Gilching, Germany), adjusted to full intensity and largest light aperture, was held directly at the level of the participant's eye, as close to the eye as possible. The participant was asked to look straight into the light and to avoid blinking. The macular stop of the ophthalmoscope was projected directly onto the macula for 30 seconds. The trained investigator looked through the viewing window of the ophthalmoscope to ensure that the red fundus reflex was visible and the corneal reflex was centered in the pupil. By continuous observation through the viewing window, the investigator controlled the fixation throughout the bleaching procedure and, in case of repeated blinking or dermatochalasis, gently opened the participant´s upper eye lid and held it in place.

Third, immediately after bleaching, PRT was assessed via a stopwatch as the seconds it took the participant to read the line below their initial VA. Measurements of PRT were stopped at the latest after 240 seconds. We analyzed PRT recorded to the nearest second for values below 241 seconds and values set to 241 when the measurement was stopped at 240 seconds without regained VA.[28]

Design of Three Independent Analyses Data Sets

Our data enabled the design of three independent data sets for cross-sectional as well as longitudinal analyses. (i) We analyzed a primary cross-sectional sample from the AugUR2 baseline assessments (2017–2019), consisting of 1316 participants eligible for PRT measurement. AugUR2 had no follow-up available. (ii) In AugUR1, PRT was introduced to the study protocol in March 2015 as part of the baseline assessment (2013–2015); for participants recruited prior to March 2015, the PRT was available

from the follow-up visit. We analyzed a second cross-sectional sample consisting of 548 individuals with PRT assessment, where no follow-up visit with AMD assessment was available. These consisted of 136 individuals with PRT assessment from the baseline visit and 412 with PRT assessment from the follow-up visit. (iii) We had further 321 independent AugUR1 participants for longitudinal analyses. They all had a PRT assessment at the baseline visit or the 3-year follow-up visit and they had their AMD status assessed at follow-up 3 years later (3- or 6-year follow-up, respectively). This enabled longitudinal analyses of PRT associations with AMD incidence within 3 years in data independent from the 2 cross-sectional analyses.

Genetic Risk Score Calculation

AugUR study participants were genotyped and genetic information was imputed as described previously.[26] In brief, based on 50 of 52 variants reported for late AMD,[27] we computed a genetic risk score (GRS) by adding the dosages of AMD risk alleles, weighed by the respective variant's published effect size.[27]

Measurement of Retinal Layer Thicknesses

We have shown previously that retinal layer thicknesses derived from optical coherence tomography (OCT) are associated with AMD cross-sectionally.[29] Thus, the structural changes in these layers related to AMD are potentially interesting to evaluate for their correlation with the functional assessment via PRT.

Thickness values of retinal layers were derived in AugUR participants as reported previously.[29] In brief, macular cube volumetric SD-OCT scans with 49 Raster lines, 20 × 20 degrees, centered on the fovea, were acquired via the Spectralis SD-OCT Plus BluePeak (Heidelberg Engineering, Heidelberg, Germany) and imported into the Heidelberg Eye Explorer 1 software (version 1.9.17.0; Heidelberg Engineering, Germany). The built-in automated segmentation of retinal layers yielded mean retinal layer thickness values from nine macular subfields determined by the ETDRS grid. For this present analysis, we analyzed mean thicknesses in the central circle (foveal, 1 mm diameter) for three layers that were associated with early or late AMD[29]: the RPE/Bruch´s membrane complex (RPE/BrM), the photoreceptor inner and outer segments including the interdigitation zone (PR-IS/OS), and the outer nuclear layer (ONL).

Data Management and Statistical Analyses

Askimed (http://www.askimed.com/) and SAS 9.4 software (SAS Institute Inc., Cary, NC, USA), were used for data management. Statistical analyses were carried out using the statistical software package IBM SPSS Statistics for Windows, version 26.0.0.1 (IBM Corp., Armonk, NY, USA).

For variable description, mean ± standard deviation or median (interquartile range [IQR]) is provided for continuous variables and % (*n*) for dichotomous variables, unless stated otherwise. To test for trend of PRT by age groups or AMD categories, we performed the nonparametric Jonckheere-Terpstra test.

Logistic regression was performed to test for associations of PRT with any, early, and late AMD status cross-sectionally, adjusted for age centered at 75 years of age, (age centered)^2, sex, and initial VA (i.e. VA before bleaching). Of note, VA decreases by age and AMD status, and PRT is correlated with VA.[11] We conducted various sensitivity analyses: (i) alternative models (i.e. fully unadjusted, without adjusting for initial VA), (ii) restricting to individuals with cataract surgery, (iii) alternative handling of extreme values, and (iv) analyzing PRT after transformation by natural logarithm. The same models were also applied to test for association of baseline PRT with incident any, early, and late AMD within 3 years.

To assess correlations of PRT with retinal layer thicknesses, we derived Spearman's rank correlation coefficients. Linear regression was conducted to test for association of the AMD-GRS with PRT (=outcome), adjusted for age centered, (age centered)^2, sex, and initial VA.

We analyzed one PRT value per person corresponding to the worse eye AMD status per person: for example, PRT was analyzed (i) for the participant´s eye which was the worse eye with regard to AMD status if both eyes were gradable for AMD, (ii) for the one eye that was gradable for AMD, or (iii) for a random eye if both eyes had the same AMD status. For longitudinal analyses, we evaluated PRT for the eye with the worse AMD status 3 years later and conducted sensitivity analyses for right and left eyes separately (with the AMD status 3 years later in the same eye).

To derive measures of diagnostic accuracy, we computed the area under the receiver operating characteristics curve (AUC) in the primary cross-sectional sample and derived the optimal cutoff for equal weight of false positive and false negative values using the Youden index. We then applied this cutoff in the independent longitudinal sample to derived positive and negative predictive values of a PRT value above

or below this cutoff, respectively, for developing AMD within 3 years.

Results

Association of PRT with AMD in the Primary Cross-Sectional Analysis

Our primary cross-sectional study sample consisted of 1222 AugUR2 participants who were eligible for PRT and AMD assessments. Of these, 1208 individuals had an available PRT value for the eye with the worse AMD status (analyzed sample; see Methods section). For 14 participants, the PRT measurement was missing, mostly due to technical reasons. Among the 1208 individuals, ages ranged from 70 to 95 years (mean age = 78.7 ± 4.8), 42.1% were men; 288 individuals had early AMD, and 78 had late AMD (Table 1). Median PRT was 91.0 seconds (IQR = 59.0–138.0; min-max = 2–241), similar between men and women, and 111 individuals had PRT values >240 seconds (see Table 1).

In these 1208 participants, PRT increased by higher age and by severity of AMD (Fig. 1A, B; Jonckheere-Terpstra test: $P < 0.0001$). We further tested PRT for association with AMD status via logistic regression adjusted for age, age^2, sex, and initial VA. We observed a statistically significant association of prolonged PRT with any, early, and late AMD compared to no AMD: odds ratios (OR) per 10 seconds of PRT and 95% confidence intervals (CIs) were 1.109 (95% CI = 1.085 to 1.132), 1.095 (95% CI = 1.070 to 1.121), and 1.165 (95% CI = 1.122 to 1.210), respectively ($P < 0.0001$; Table 2).

To document robustness of the association results, we performed various sensitivity analyses. (i) We explored associations via logistic regression and alternative adjustments: without adjustment, or adjusted for age, age^2, and sex. We found similar ORs and P values (Supplementary Table S1; e.g. for any AMD: unadjusted OR = 1.117 [1.094 to 1.140, $P < 0.0001$ versus OR = 1.109 [1.085 to 1.132, $P < 0.0001$ from the original model). (ii) We evaluated PRT and its association with AMD restricting to the 437 individuals who reported a previous cataract surgery in the respective eye, because PRT might have been influenced by

Table 1. Participant Characteristics of Primary Cross-Sectional Analysis

	All ($n = 1208$)	Men ($n = 509$)	Women ($n = 699$)
Age, y, mean ± SD	78.7 ± 4.8	78.4 ± 4.9	79.0 ± 4.7
PRT, s[a], median (IQR)	91.0 (59.0–138.0)	91.0 (59.0–138.0)	92.0 (60.0–138.0)
AMD status[b]			
No AMD, % (*n*)	69.7 (842)	73.3 (373)	67.1 (469)
Mild early AMD, % (*n*)	9.6 (116)	8.4 (43)	10.4 (73)
Moderate early AMD, % (*n*)	6.9 (83)	4.9 (25)	8.3 (58)
Severe early AMD, % (*n*)	7.4 (89)	7.1 (36)	7.6 (53)
Late AMD, % (*n*)	6.5 (78)	6.3 (32)	6.6 (46)
VA [logMAR][c], median (IQR)	0.1 (0.1–0.3)	0.1 (0.0–0.3)	0.1 (0.1–0.3)
Cataract surgery[d], % (*n*)	36.4 (437)	30.2 (153)	40.9 (284)
GRS [weighted][e], mean ± SD	14.3 ± 1.2	14.4 ± 1.2	14.3 ± 1.2

Shown are age, PRT, AMD status, VA, history of cataract surgery, and GRS for the 1208 analyzed AugUR2 baseline participants, separated for men and women.

SD, standard deviation; IQR, interquartile range (25th to 75th quartile); PRT, photostress recovery time; AMD, age-related macular degeneration; VA, visual acuity; logMAR, Logarithm of the Minimum Angle of Resolution; GRS, genetic risk score.

[a]PRT per person is given for the eye with the more severe AMD stage, a random eye if both had the same AMD stage, or the one available eye if only one eye was gradable for AMD. For the 111 participants that did not reach required VA after 240 seconds, values were set to 241 seconds.

[b]AMD grading was performed on color fundus images following the Three Continent AMD Consortium Severity Scale.[5] AMD status per person was derived as the AMD status of the eye with the more severe stage ("worse eye") when both eyes were gradable, or as the status of the one available eye otherwise.

[c]Here, given is the initial VA in logMAR before bleaching, measured via standard ETDRS charts in 4 meter distance with participants´ own correction if applicable.

[d]History of cataract surgery was assessed via interview-based questionnaire.

[e]GRS was available for 1178 AugUR2 baseline participants, 501 men and 677 women.

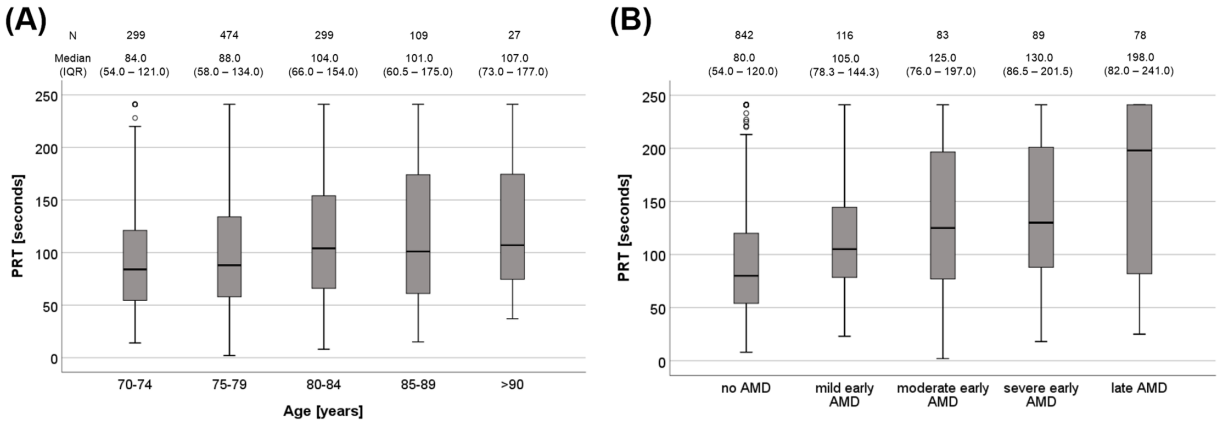

Figure 1. **Distribution of PRT in cross-sectional analysis.** Shown are median, 25th and 75th percentile (box) of PRT values by (**A**) age groups and (**B**) AMD disease stages in AugUR2 participants. The increase of PRT by higher age groups and by worse AMD stages was statistically significant (Jonckheere-Terpstra test, both $P < 0.0001$).

cataract: median PRT [IQR] was indeed higher at 93.0 [IQR = 62.0–136.0] seconds (compared to 91.0 [IQR = 58.0–138.0] seconds in those without cataract surgery). In these 437 individuals, we found the same association of prolonged PRT with any, early or late AMD with similar OR (OR per 10 seconds = 1.111, 1.093, and 1.171, respectively; $P < 0.0001$; Supplementary Table S2). (iii) Using the PRT values of 241 seconds for the

Table 2. Associations of PRT With AMD

	Any AMD[a]		Early AMD[a]		Late AMD[a]	
	OR [95% CI]	*P* Value	OR [95% CI]	*P* Value	OR [95% CI]	*P* Value
(A) Primary analysis						
PRT (per 10 seconds)[b]	1.109 [1.085, 1.132]	**$1.06*10^{-21}$**	1.095 [1.070, 1.121]	**$9.67*10^{-15}$**	1.165 [1.122, 1.210]	**$4.05*10^{-15}$**
Age (centered)	1.060 [1.000, 1.124]	**0.05**	1.074 [1.009, 1.144]	**0.03**	0.992 [0.882, 1.116]	0.90
Age centered^2	1.000 [0.996, 1.005]	0.84	0.999 [0.995, 1.004]	0.81	1.005 [0.997, 1.013]	0.20
Sex (0 female, 1 male)	0.761 [0.580, 0.998]	**0.05**	0.709 [0.530, 0.948]	**0.02**	1.054 [0.613, 1.812]	0.85
VA (per 0.1 logMAR)[c]	1.109 [1.030, 1.193]	**0.01**	1.001 [0.920, 1.089]	0.98	1.484 [1.310, 1.681]	**$5.91*10^{-10}$**
(B) Replication analysis						
PRT (per 10 seconds)[b]	1.074 [1.042, 1.107]	**$4.00*10^{-6}$**	1.067 [1.032, 1.103]	**$1.40*10^{-4}$**	1.113 [1.048, 1.181]	**$4.51*10^{-4}$**
Age (centered)	0.984 [0.892, 1.086]	0.75	0.973 [0.877, 1.081]	0.61	1.022 [0.832, 1.255]	0.84
Age centered^2	1.003 [0.996, 1.009]	0.40	1.003 [0.996, 1.010]	0.38	1.003 [0.991, 1.015]	0.65
Sex (0 female, 1 male)	1.164 [0.763, 1.777]	0.48	1.117 [0.710, 1.759]	0.63	1.397 [0.583, 3.349]	0.45
VA (per 0.1 logMAR)[c]	1.157 [1.033, 1.296]	**0.01**	1.062 [0.935, 1.206]	0.36	1.523 [1.255, 1.849]	**$2.10*10^{-5}$**

(**A**) We analyzed associations of PRT with any, early, or late AMD in the 1,208 AugUR participants of the primary cross-sectional analysis (AugUR2 baseline). We used logistic regression adjusted for baseline age, age^2, sex and baseline initial visual acuity before bleaching. Shown are odds ratios, 95% confidence intervals, and *P* values for any AMD versus no AMD ($n = 366$ vs. 842), early AMD versus no AMD ($n = 288$ vs. 842), and late AMD versus no AMD ($n = 78$ vs. 842). (**B**) Replication analysis in 486 individuals of an independent cross-sectional study sample (AugUR1) revealed similar results: shown are odds ratios, 95% confidence intervals, and *P* values for any AMD versus no AMD ($n = 152$ vs. 363), early AMD versus no AMD ($n = 107$ vs. 363), and late AMD versus no AMD ($n = 45$ vs. 363).

AMD, age-related macular degeneration; OR, odds ratio; CI, confidence interval; PRT, photostress recovery time; VA, visual acuity; logMAR, logarithm of the minimum angle of resolution.

[a]AMD grading was performed on color fundus images following the Three Continent AMD Consortium Severity Scale.[5] AMD status per person was derived as the AMD status of the eye with the more severe stage ("worse eye") when both eyes were gradable, or as the status of the one available eye otherwise.

[b]PRT per person is given for the eye with the more severe AMD stage, a random eye if both had the same AMD stage, or the one available eye if only one eye was gradable for AMD. For 111 participants that did not reach required VA after 240 seconds, values were set to 241 seconds.

[c]Here, given is the initial VA in logMAR before bleaching, measured via standard ETDRS charts in 4 meter distance with participants´ own correction if applicable.

111 participants who did not reach the required VA after 240 seconds (see the Methods section) lead to a peak of PRT values at 241 seconds. To rule out any statistical artifact by this peak, we performed a sensitivity analysis by randomly distributing PRT values of these 111 individuals to values ≥241 seconds following the overall distribution. We also observed 4 individuals with PRT values <10 seconds and set these to 10 seconds for this analysis. We found similar association results (Supplementary Tables S3A, S3B, S3C). (iv) We repeated the analyses from (iii) using the natural logarithm of these PRT values and observed, again, the same associations (Supplementary Tables S3D, S3E, S3F).

To correlate quantitative PRT with quantitative measurements of retinal structure, we derived the Spearman correlation coefficient of PRT and OCT-derived thicknesses of three retinal layers, which had previously been shown to be associated with AMD (increased thickness of RPE/BrM, decreased thickness of PR-IS/OS and ONL for individuals with AMD).[29] We found a directionally consistent, but small correlation of PRT and RPE/BrM as well as PR-IS/OS (Spearman correlation coefficient $r = 0.06$, $P = 0.03$, and $r = -0.17$, $P < 0.0001$, respectively). We found no correlation for PRT and ONL ($r = -0.002$, $P = 0.93$).

To further substantiate the link between PRT and AMD, we tested the GRS for AMD for association with PRT cross-sectionally in the 1178 of the 1208 participants who also had genetic data. Using the natural logarithm of PRT as outcome in a linear regression model adjusted for age, age^2, sex, and initial VA, we found a highly significant association of the AMD-GRS with PRT ($P = 7.70*10^{-11}$). This further emphasizes the notion of common mechanisms for AMD and prolonged PRT that are independent of age.

Association of Prolonged PRT With AMD Status was Replicated in Independent Cross-Sectional Data

To replicate our finding of prolonged PRT being associated with AMD in cross-sectional data, we analyzed our second cross-sectional sample (i.e. AugUR1 participants with PRT measurement who did not have a 3-year follow-up thereafter; see the Methods section). This included 486 participants who had a valid PRT value for the eye with the worse AMD status. The participant characteristics of these individuals were comparable to those in the primary cross-sectional analysis (Supplementary Table S4). We were able to confirm the significant association of prolonged PRT with any, early, and late AMD with similar OR estimates: ORs (95% CI) per 10 seconds = 1.074 (95% CI = 1.042, 1.107), 1.067 (95% CI = 1.032, 1.103), or 1.113 (95% CI = 1.048, 1.181), respectively ($P < 0.0001$; see Table 2, Supplementary Table S5).

Prolonged PRT was Associated With Incident AMD in Independent Longitudinal Study Data

Following our observation of consistent PRT associations with AMD status cross-sectionally in two independent data sets, we were interested whether PRT was just a marker of concurrent AMD or also predictive for AMD development. In longitudinal data, we evaluated the PRT from individuals without any AMD for association with incident any, early, and late AMD ascertained in the follow-up 3 years later. For this, we analyzed the 233 AugUR1 participants with valid PRT measurement and ascertained lack of AMD (from baseline or 3-year follow-up), for whom the AMD status was available at the follow-up 3 years later (3- or 6-year follow-up, respectively; see the Methods section; mean follow-up time 3.2 ± 0.2 years, 32 individuals with incident early AMD, and 5 with

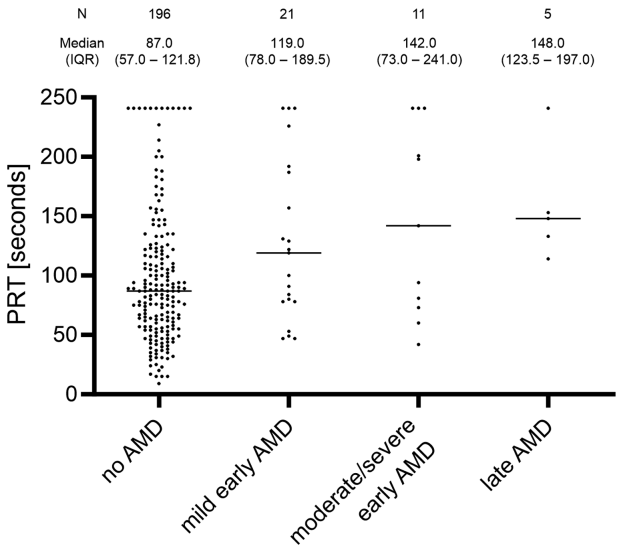

Figure 2. Distribution of PRT by incident AMD disease stages. Shown are dot plots with median of PRT measured among 233 AugUR1 participants without AMD by their AMD disease stage at the 3-year follow-up. We observed 32 participants with incident early AMD and 5 individuals with incident late AMD. As there was only one participant with incident severe early AMD, moderate and severe incident AMD were collapsed into one group. The increase of PRT by worse incident AMD disease stages was statistically significant (Jonckheere-Terpstra test, $P = 0.0006$).

Table 3. Associations of PRT With Incident AMD in the Longitudinal Analysis

	Any AMD[a]		Early AMD[a]		Late AMD[a]	
	OR [95% CI]	*P* Value	OR [95% CI]	*P* Value	OR [95% CI]	*P* Value
PRT (per 10 seconds)[b]	1.112 [1.049, 1.178]	**$3.69*10^{-4}$**	1.100 [1.035, 1.169]	**0.002**	1.162 [1.008, 1.339]	**0.04**
Age (centered)	1.004 [0.881, 1.144]	0.95	0.978 [0.855, 1.119]	0.74	1.414 [0.750, 2.667]	0.28
Age centered^2	0.999 [0.986, 1.012]	0.87	1.001 [0.989, 1.014]	0.82	0.961 [0.889, 1.038]	0.31
Sex (0 female, 1 male)	2.373 [1.092, 5.160]	**0.03**	2.138 [0.953, 4.797]	0.07	4.796 [0.465, 49.428]	0.19
VA (per 0.1 logMAR)[c]	0.922 [0.723, 1.176]	0.51	0.883 [0.676, 1.152]	0.36	1.243 [0.716, 2.160]	0.44

We analyzed PRT measured in 233 AugUR participants without AMD at baseline for the association with incident any, early, or late AMD ascertained three years later (AugUR1 participants with 3-year follow-up information on AMD status independent of the cross-sectional data analyses) using logistic regression adjusted for baseline age, age^2, sex, and baseline initial visual acuity before bleaching. Shown are odds ratios, 95% confidence intervals, and *P* values for incident any AMD versus no AMD at 3-year follow-up (37 incident cases, 196 no AMD), incident early AMD versus no AMD at 3-year follow-up (32 incident cases, 196 no AMD), and incident late AMD versus no AMD at 3-year follow-up (5 incident cases, 196 no AMD).

AMD, age-related macular degeneration; OR, odds ratio; CI, confidence interval; PRT, photostress recovery time; VA, visual acuity; logMAR, logarithm of the minimum angle of resolution.

[a]AMD grading was performed on color fundus images following the Three Continent AMD Consortium Severity Scale.[5] AMD status per person was derived as the AMD status of the eye with the more severe stage ("worse eye") when both eyes were gradable, or as the status of the one available eye otherwise.

[b]PRT per person is given for the eye with the more severe AMD stage, a random eye if both had the same AMD stage, or the one available eye if only one eye was gradable for AMD. For 19 participants that did not reach required VA after 240 seconds, values were set to 241 seconds.

[c]Here, given is the initial VA in logMAR before bleaching, measured via standard ETDRS charts in 4 meter distance with participants´ own correction if applicable.

incident late AMD). These individuals were independent from, but comparable to, the individuals in the two previous cross-sectional analyses (Supplementary Table S6).

Median PRT was 129.0 seconds (IQR = 79.0–199.5) for the 37 individuals with incident early or late AMD 3 years later and 87.0 seconds (IQR = 57.0–121.8) for the 196 individuals who remained AMD-free. Median PRT increased by severity of AMD status observed 3 years later (Fig. 2; Jonckheere-Terpstra test $P = 0.0006$).

When applying logistic regression adjusted for age, age^2, sex, and initial VA, we found a significant association of prolonged PRT with incident any, incident early AMD, and incident late AMD (Table 3): OR per 10 seconds (95% CIs) = 1.112 (95% CI = 1.049, 1.178), 1.100 (95% CI = 1.035, 1.169), or 1.162 (95% CI = 1.008, 1.339), respectively ($P = 0.0004$, 0.002, or 0.04, respectively). Sensitivity analyses without adjustment or only adjusted for sex, age, and age^2 yielded similar results (Supplementary Table S7). In addition to these analyses using the eye that showed the worse AMD status at follow-up 3 years later, sensitivity analyses using the right (left) eyes with the respective PRT and AMD status showed the same results for any and early AMD (Supplementary Table S8). Despite the limited sample size of individuals with incident AMD, these statistically significant results suggest prolonged PRT as a predictive biomarker for the development of late and early AMD.

To assess the diagnostic accuracy of PRT, we derived receiver operating curves and AUC of age, PRT, and both combined for any, early, or late AMD based on the primary cross-sectional sample (n = 1208). The AUC of PRT was higher than that of age; the AUC of PRT and age combined was 0.71, 0.69, and 0.79 for any, early, or late AMD, respectively (Figs. 3A–C). The Youden-index-based optimal cut-off for PRT values were 112.5 seconds, 102.5 seconds, and 197.5 seconds for any AMD, early AMD, or late AMD, respectively. In the independent longitudinal sample (n = 233, including 37 individuals with incident any AMD and 32 with early AMD; not specifying this for incident late AMD due to limited numbers), the positive and negative predictive values of PRT above or below this cutoff, respectively, were 27.8% and 90.3% to predict development of any AMD 3 years later and 19.8% and 89.4% for early AMD.

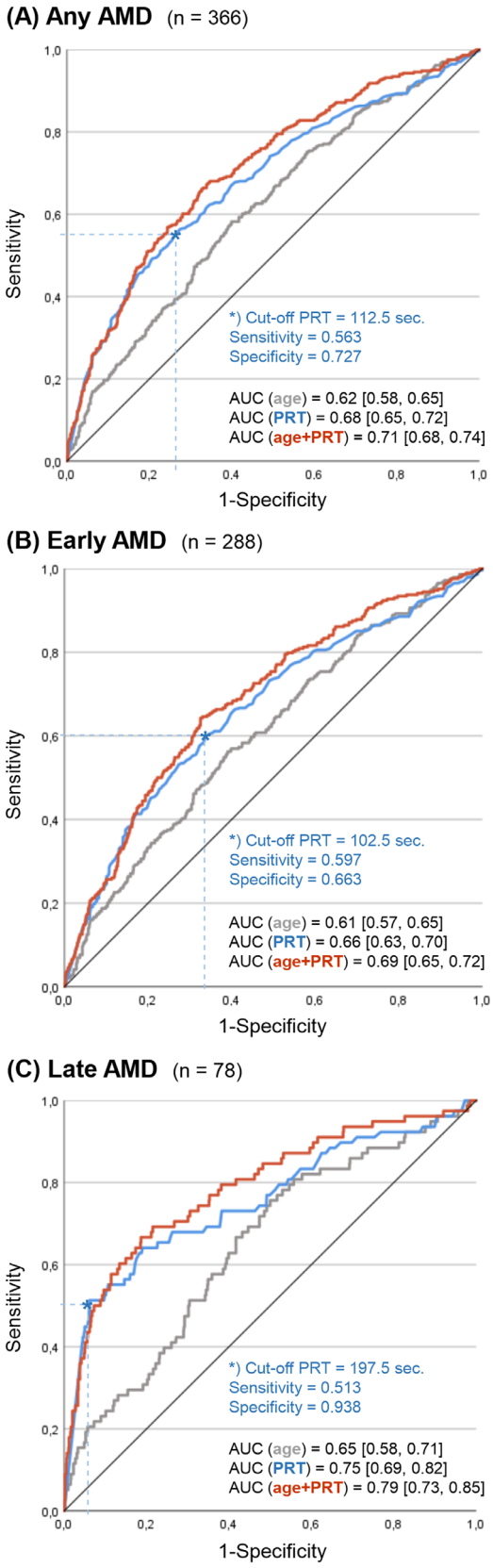

Figure 3. **Diagnostic accuracy of PRT.** Shown are receiver operating curves and the corresponding areas-under-curve (AUC) for age, PRT, and the combination of age + PRT, in (**A**) any AMD, (**B**) early AMD, and (**C**) late AMD, based on the 1208 AugUR2 participants of our primary cross-sectional data. Also shown is the Youden-index-based optimal cutoff PRT value as well as sensitivity and specificity at this value.

Discussion

Here, we present a systematic evaluation of PRT and its association with AMD in three independent data sets from a population-based cohort of the mobile elderly aged 70+ years. We report highly significant associations of prolonged PRT with any, early as well as late AMD in a primary cross-sectional data set of approximately 1200 individuals and robust results in sensitivity analyses. Moreover, we confirmed these associations in independent cross-sectional replication data. Importantly, we found significant association of PRT with incident AMD in a third independent study sample with 3-year follow-up after PRT measurement. Our observation of a prolonged PRT among initially AMD-free individuals who showed signs of early or late AMD on color fundus photography 3 years later suggests PRT as predictive biomarker for early and late AMD, independent of age and VA.

Several tests of retinal function in AMD have been established, especially for clinical trials.[7–9] However, these tests are all quite time consuming, expensive, as they require special equipment, and strenuous for the persons undergoing the tests (e.g. sitting in the dark for up to 45 minutes when testing RMDA).[9] Thus, they are not easily implementable in epidemiological studies, which typically have a broad and extensive study program to cover a variety of diseases and aspects. We therefore chose to test cone-mediated adaptation after glare using the photostress test with an established protocol, as described by Margrain et al.[12] This protocol had been shown to be least prone to variability and closest to standardized reference techniques, compared to other methods using less sophisticated light sources and shorter bleaching times.[12] Margrain and colleagues analyzed 50 healthy subjects aged 21 to 69 years and reported PRT to increase by age with model-based predicted values of PRT ranging from 30 to 80 seconds in individuals aged 65 to 69 years.[12] Their mean overall PRT of 50.2 ± 13.0 seconds was lower than the mean of 84.5 ± 43.2 seconds among our participants without retinal disease or cataract, as expected, due to the higher age of our participants (70+ years). Our study thus contributes reference PRT values in a particularly old population.

Importantly, PRT has not yet been evaluated in population-based studies and systematic data on PRT is generally scarce. A previous cross-sectional hospital-

based study of 2104 participants aged 50 to 75 years included 37 patients with any AMD[17]; PRT was found to be prolonged in eyes with early AMD, normal VA, and normal Amsler Grid test compared to AMD-free individuals.[17] Another cross-sectional hospital-based study compared 221 patients with early AMD to 109 healthy controls, mean age 73 years, and suggested PRT to be potentially clinically useful to quantitatively assess retinal function in early AMD.[16] These hospital-based study results are in line with our cross-sectional population-based results.

One previous cross-sectional study has reported increased PRT in the fellow eyes of 133 patients with unilateral neovascular AMD.[11] The authors state that nearly half of those 133 fellow eyes with a normal VA exhibited delayed PRT and suggested that delayed PRT was an early manifestation of the disease process. Their result also suggests that prolonged PRT and AMD have common person-specific risk factors. Our observed association of the AMD genetic risk score with PRT substantiates a common etiology that is beyond old age or acquired risk factors.

Whereas these and our cross-sectional results highlight PRT as a biomarker for retinal function in early AMD, any longitudinal evaluation on PRT among AMD-free individuals for association with incident AMD had been lacking. Our study is the first providing longitudinal data on PRT with incident AMD diagnosed on color fundus images and the first evidence for PRT to be a predictive biomarker for early and late AMD development. Our results are in line with published longitudinal data suggesting delayed RMDA as a predictive functional biomarker for incident early AMD in 325 patients with a 3-year follow-up.[10] Our results on PRT and these previous results on RMDA support the hypothesis that delayed adaptation is indicative of an impaired visual cycle due to cell damage in photoreceptors that is not yet structurally visible as AMD (e.g. on color fundus photography). Both RMDA and PRT measure the efficiency of the visual cycle and might therefore be delayed already before structural changes are detectable[9] – hence, the association with incident AMD. Whereas RMDA assesses the rod dominated para- and perifovea, PRT measures the cone dominated fovea, the latter being affected the most by AMD.[1,9] Because cones have been shown to be more resilient than rods,[30] changes in RMDA might be detectable even before changes in PRT become apparent. However, longitudinal AMD studies which compare RMDA to PRT are missing.

The biological link between PRT and visual cycle efficiency is further supported by the correlation of the PRT with the OCT-derived thickness of the PR-IS/OS layer, where the visual cycle predominantly takes place. The negative correlation is in line with a previously described thinning of the PR-IS/OS in early AMD cross-sectionally.[29] Further studies are warranted that include multimodal imaging for AMD grading and investigate PRT association with AMD features detected on OCT or fundus autofluorescence. Some individuals that are graded as "no AMD" using color fundus imaging might reveal very early, subclinical AMD features on other imaging modalities. The question remains if prolonged PRT is associated with incident early AMD also for participants without these subclinical changes at baseline.

Our results might be informative for clinical studies, where quantitative functional outcome measures for early AMD stages are highly sought after.[8] Clinical studies on AMD development might benefit from excluding individuals with short PRT to enhance the probability of an event and thus power. The high negative predictive value of 90.3% suggests that individuals aged at least 70 years with a PRT <112 seconds will likely not develop AMD within 3 years. Future work might explore the value of PRT also for clinical routine to help plan the interval to the next ophthalmology practitioner visit.

Our study is large, population-based, with standardized protocols across all surveys and follow-up. All color fundus images were manually graded for AMD according to the well-established 3CACSS[5] by one experienced ophthalmologist with double grading by an additional independent grader as published previously.[6,26] Some limitations need to be acknowledged. We have only one measurement of PRT per participant and time point. Therefore, we cannot provide internal study data on reliability and repeatability. Repeated measurements were not feasible in the AugUR study setting, which was tailored to meet the requirements of old-aged study participants. Repeated bleaching within one visit cannot be expected to yield valid results. Repeated visits within a short time interval are a burden on elderly study participants. The primary source of variability is how much photopigment is bleached, and this depends on the bleaching method. The protocol we have chosen is a high intensity and long duration strategy, which ensures the bleaching of almost all cone photopigment. Hence, variations, for example, in bleach intensity become less relevant. Importantly, the use of a direct ophthalmoscope allows for a control of fixation.[12] Moreover, the simplicity of this PRT protocol can be considered a strength, making it easily implementable in large population-based cohort studies as well as clinical settings which have limited access to diagnostic tools.

One might assume that cataract altered PRT, as it, for example, potentially scatters the light during

bleaching of the macula and/or reduces initial VA in general. Previous work showed that cataract[31] or cataract surgery (Perez–Carrasco et al., 06-A-3649-ARVO, poster presented 2006) had no effects on PRT, whereas others excluded patients with cataract from analyses.[32] Our large study data in individuals aged 70+ years, where presence of cataract is particularly common, allowed us to perform sensitivity analyses focusing on patients after cataract surgery: we found the same associations. Of note, pupil size has been reported to not significantly influence PRT.[12]

A challenge for PRT association analyses with AMD is the prolonged PRT by increased age, which can result in association by confounding, because AMD frequency substantially increases by age as well. We adjusted our analyses not only by a linear age term, but also by a squared age term, which would account for complex dependencies on age. Importantly, we showed that a previously established genetic risk score for AMD,[26,27] which strongly predicts AMD development virtually from the cradle, also predicted PRT. This makes a pure confounder effect by age rather unlikely.

In summary, our population-based cohort data from the mobile elderly aged 70+ years provides evidence of a stable, highly significant association of prolonged PRT with early as well as late AMD cross-sectionally and, most importantly, also longitudinally. Our novel data and results thus highlight the potential of PRT not only as an easily measurable, quantitative biomarker for disturbed photoreceptor function due to AMD, but also as a predictive marker for the development of early as well as late AMD. Further studies are warranted to substantiate its merit also in clinical routine or clinical studies.

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* KJS and IMH supervised these analyses jointly.

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