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Longitudinal Evaluation of Visual Function Impairments in Early and Intermediate Age-Related Macular Degeneration Patients

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Purpose: To evaluate visual function (VF) changes in early and intermediate age-related macular degeneration (eAMD and iAMD) over 24 months.

Design: Prospective, observational natural history study.

Participants: Participants were enrolled at the Duke Eye Center.

Methods: A total of 101 subjects (33 with eAMD, 47 with iAMD, and 21 normal controls) were recruited. Visual function (VF) tests included best-corrected visual acuity (BCVA), low- luminance visual acuity (LLVA), microperimetry (MP), cone contrast tests (CCTs), and dark adaptation (DA). Mixed-effect model repeated measures based on absolute values and change from baseline identified VF tests differentiating AMD from controls and revealing longitudinal VF decline when controlling for covariates (baseline value, age, coronary artery disease, dry eye, and phakic status). Nine AMD genetic risk variants, combinations of these (genetic burden score), reticular pseudodrusen (RPD), and hyperreflective foci (HRF) were tested as predictors of diagnosis and VF performance.

Main Outcome Measures: Longitudinal changes in VF metrics over 24 months.

Results: A total of 70 subjects completed the 2-year visit (22 with eAMD, 31 with iAMD, and 17 controls). Percent reduced threshold (PRT) on MP and CCT red significantly distinguished iAMD versus controls after 12 and 24 months, respectively. Cone contrast test red, PRT, and absolute threshold (AT) on MP showed significant longitudinal deterioration of VF in iAMD versus baseline at 12 months and onward, however, with a reduced rate of worsening. The DA data confirmed a preexisting functional deficit in iAMD at baseline and revealed an increasing proportion of poorly performing iAMD subjects in DA over the study period. None of the other VF measures showed consistent significant changes among the normal, early, and intermediate groups or over time. The genetic burden score was significantly associated with AMD diagnosis (relative risk for iAMD $= 1.64$, $P < 0.01$) and DA ($r = 0.42$, $P = 0.00005$). Reticular pseudodrusen and HRF showed moderate associations with DA and weak to moderate associations with MP variables.

Conclusions: In iAMD, MP variables, CCT red, and DA revealed slow and nonlinear functional decline over 24 months. A structure–function relationship in eAMD and iAMD stages was demonstrated among HRF, RPD, and DA, possibly modified by genetic risk factors. These structural and functional features represent potential end points for clinical trials in iAMD. *Ophthalmology Science 2022;2:100173* © *2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material available at www.ophthalmologyscience.org.

Age-related macular degeneration (AMD) is one of the leading causes of central vision loss in individuals aged more than 50 years in developed countries and is responsible for 8.7% of blindness worldwide.[1] Clinically, AMD stages are determined by the Age-Related Eye Disease Study (AREDS) classification.[2] Although the majority of patients diagnosed with AMD are affected by the nonexudative or dry form of AMD, the only currently Food and Drug Administration–approved therapies available are targeting anti-vascular endothelial growth factor for the exudative or neovascular form.

A paucity of biomarkers to aid in monitoring disease progression in the early stages of AMD has proven to be an obstacle for therapeutic development in dry AMD. Currently, ophthalmologists use a number of psychophysical tests to measure visual acuity and structural changes in the retina. However, there is a need for more sensitive tests that are effective in detecting significant changes of progression from early AMD (eAMD) to intermediate AMD (iAMD), as well as progression of iAMD to the late forms of AMD (neovascular AMD and geographic atrophy). Prior work has characterized cross-sectional differences in visual function (VF) metrics such as color vision,[3-7] low-luminance deficit (LLD),[8] a tablet-based retinal function,[9] electroretinogram measures,[6,8,10,11] static and flicker perimetry,[12] microperimetry (MP),[13-19] and dark

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adaptation (DA)[20-22] between normal individuals and those with early to intermediate stages of AMD. Ours was a large-scale longitudinal study with the goal to explore a comprehensive battery of functional biomarkers in early stages of AMD, preceding more recent extensive efforts such as the MACUSTAR study in Europe.[23,24]

Our group previously showed the feasibility of using the VF test to evaluate AMD subjects and has reported baseline and 12-month follow-up findings on VF impairments in eAMD and iAMD subjects.[20-22] Briefly, we found that low-luminance visual acuity (LLVA), MP with its 2 variables, percent reduced threshold (PRT), absolute threshold (AT), cone contrast test (CCT), and DA could serve as functional measures differentiating among normal, non-AMD, and iAMD stages[20] and that MP and CCT can detect functional progression of dry AMD within a period as short as 12 months.[21] In this prospective, longitudinal, observational study, we evaluated our primary hypothesis that the selected VF assessments (best-corrected visual acuity [BCVA], LLVA, CCT, MP, and DA) may be suitable functional biomarkers to describe disease progression in dry eAMD and iAMD over a period of 24 months. We used a mixed-effect repeated measure (MMRM) model to control for covariates (baseline value, age, coronary artery disease, dry eye, and cataract status) and to explore the cross-sectional performance of the different assessments at 6-month intervals and any longitudinal VF change over the period of 24 months. A second goal was to assess whether known genetic risk alleles for AMD onset or progression, a combined genetic burden score, or 2 imaging biomarkers at baseline (presence or absence of reticular pseudodrusen [RPD] or hyperreflective foci [HRF]) on multimodal (spectral domain OCT [SD-OCT], infrared, and auto-fluorescence) images may be predictors of AMD diagnosis at baseline or VF performance at the different time points.

Methods

Study Participants

The inclusion of study participants in a single-center, prospective, longitudinal, observational study of eAMD (ClinicalTrials.gov identifier, NCT01822873), named "Duke study of **F**unctional **E**ndpoints for **A**ge-rela**t**ed Mac**u**la**r** D**e**generation" (Duke FEATURE), has been previously described.[20,21] Subjects with eAMD and iAMD were enrolled from the Duke Eye Center patient population, and control participants were enrolled from the Duke Optometry and Comprehensive Eye Clinics or were family members or friends of the AMD patients. At the baseline visit, participants were classified using color fundus photographs (CFPs) into the following categories: healthy control (AREDS category 1), eAMD (AREDS category 2), and iAMD (AREDS category 3).[2] Healthy control participants presented with fewer than 5 small drusen that measured less than 63 μm and no other signs of AMD in either eye. Subjects with multiple small drusen (63−124 μm in diameter), retinal pigment epithelium (RPE) abnormalities, or both were classified into the eAMD group. Those with multiple intermediate drusen and at least 1 large drusen (> 125 μm) were classified into the iAMD group.[2] All patients had at most mild cataracts that were not visually significant or were after cataract extraction. At baseline, presence of cataract was observed in 6 (29%) normal, 12 (37%) early, and 19 (40%) intermediate study eyes. Exclusion criteria included evidence of retinal hard exudates, neovascular pathology, geographic atrophy of the RPE and choriocapillaris, sensory retina or RPE detachment, subretinal or sub-RPE fibrovascular proliferation, a disciform scar in either eye, or visually significant cataracts. From the recruited 101 elderly subjects at baseline,[20] 70 were retained at 24 months. Among these participants, 17 were healthy control subjects, 22 were eAMD subjects, and 31 were iAMD subjects.

This clinical study, approved by the Duke University Health System Institutional Review Board, was conducted in accordance with Good Clinical Practice following the guidance documents and practices offered by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use or applicable international regulatory authority laws, regulations, and guidelines. All patients signed a written informed consent before testing. This study abides by the tenets of the Declaration of Helsinki.

Imaging and VF Testing

After the baseline evaluation, 6-, 12-, 18-, and 24-month follow-up assessments were performed according to the standard-of-care clinic visits for AMD participants. Participants underwent imaging evaluation with stereo CFP (Zeiss FF 450 Plus IR; Carl Zeiss Meditec, Inc.), fundus autofluorescence (Spectralis 2; Heidelberg Engineering GmbH), and SD-OCT (Spectralis 2; Heidelberg Engineering).[17] Retinal imaging was followed by clinical examinations by Duke Eye Center ophthalmologists, and a medical retinal specialist (EML) evaluated pigmentary changes status and drusen size. Presence or absence or RPD on SD-OCT, infrared and autofluorescence, and HRF on SD-OCT was recorded for baseline images. Hyperreflective foci were defined as hyperreflective lesions on OCT detected within the retina, adjacent to the RPE layer, or adjacent to the inner border of drusen. These lesions were focal, well-circumscribed, hyperreflective (i.e., reflectivity greater than the RPE layer), and clearly distinguishable from cross-sections of retinal blood vessels, as previously described.[25,26] If patients showed signs of progression to a different stage of AMD, they were removed from the trial. Additionally, demographics, ocular and medical history, and full ophthalmic examination results of each study subject were recorded.

Visual function tests performed at baseline and every 6 ± 2 months thereafter included ETDRS; BCVA;[6,7] LLVA; CCT red, green, and blue; MP; and DA.[20,21] For LLVA evaluation, study subjects read the ETDRS chart (luminance 160 cd/m^2) through a 2.0-log neutral density filter that reduces luminance by 100-fold.[27] Low luminance deficit (LLD) was calculated as the difference between BCVA and LLVA in ETDRS letters. The CCT (Innova Systems) tested for deficits in color discrimination[22] by individual cone types by asking participants to select colored letters identified by a single cone type (long-, medium-, or short-wavelength photoreceptors for red, green, and blue colors, respectively) against a gray background with luminance of 21.5 cd/m^2. To determine the threshold for distinguishing color, the letters were presented in descending order of color contrast. Color deficiency was determined if the CCT results scored below 75% on a 100-point scale, and 90% to 100% represented normal cone function.

During MP testing (Macular Integrity Assessment; Center Vue), retinal sensitivity was measured using the standard 10 degree (37 stimuli) Macular Integrity Assessment grid after dilation with tropicamide 1% and phenylephrine 2.5%. This grid consisted of 37 test loci distributed in a radial pattern, sampling retinal locations at 0° and 1°, 2°, and 3° eccentricity from the fovea. Goldman III achromatic stimuli with stimulus duration of 200 ms were

Table 1. Number of Patients at Each Visit

Month	Normal	Early	Intermediate	Total
0	21	33	47	101
6	17	30	40	87
12	19	27	40	86
18	15	20	32	67
24	17	22	31	70

presented on a dim white background (1.27 cd/m^2) one at a time. The test strategy was 4-2 staircase. Microperimetry results were reported in 2 categories: percent reduced threshold (PRT) and absolute threshold (AT). Percent reduced threshold was defined as the percentage of loci across the grid with retinal sensitivity values below the normal level of 25 decibels (dB), whereas AT was defined as the average of retinal sensitivity values (in dB) from all loci tested using the grid.

To decrease DA testing time, DA was measured on the dilated study eye using the AdaptDx dark adaptometer (MacuLogix) with a modified protocol for patients with iAMD, as previously described.[20] Corrective lenses were used to correct for blur in the study eye, whereas the fellow eye was occluded. To avoid bleaching errors, the study coordinators used the "Demonstration" test in the beginning of each DA testing session for each eye (as recommended by the AdaptDx manual of procedures) and were in close contact with the AdaptDx technical team for any questions or need to troubleshoot. Using an infrared camera, the operator centered the study eye to the fixation light (635 nm). The tested eye was subjected to a 505-nm photoflash (0.8-ms duration, 1.8 $\times$ 10^4 scot cd/m^2 s intensity), equivalent to 76% bleaching level for rods.[20] The flash of light bleached a 2° area of the retina centered at 5° on the inferior visual meridian.[28] Immediately after bleaching, the subject began sensitivity measurements. While focusing on the fixation light, the subject pushed a handheld button when a stimulus light (505-nm, 2° circular test spot at 5° on the inferior visual meridian) was visible. The initial stimulus light intensity was 5 scot cd/m^2 and was decreased in steps of 0.3 log units until the subject stopped responding. If the subject indicated that the stimulus was not visible, the intensity was increased in 0.1 log unit steps until the subject detected the stimulus, which was then defined as the threshold. The subject received a 15-second rest period between threshold measurements. Subsequent threshold measurements started with an intensity stimulus that was 0.2 log units brighter than the prior. If a large deviation was noted on a threshold from the prior threshold, a fixation error was recorded, and an additional threshold was measured. Dark adaptation testing was stopped when the subject's sensitivity was twice consecutively measured to be greater than 5 $\times$ 10-3 scot cd/m^2 or the test duration reached 20 minutes, whichever was sooner. The 20 minutes was chosen to decrease testing burden in our aging participants. Immediately after each DA test at each visit, the testing results were reviewed to ensure fixation error rates $<$ 30% and absence of AdaptDx warnings denoting bleaching errors.

Data Management and Statistical Analyses

Data on demographics, comorbidities, and results of the VF assessments were collected from case report forms and double-entered into the Research Electronic Data Capture database by certified data entry analysts from the Duke Office of Clinical Research. The double data entry for the whole study was finalized after completion of the study in its entirety. The current final dataset also contains information for covariates that was added since our prior publications.[20] The necessary sample size was determined before the start of the study through a power calculation based on LLVA values obtained in our pilot study,[22] as previously described.[20]

The data analysis for this work was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows (2002−2012 SAS Institute Inc.) The reasons for study discontinuation of study participants and timing of study discontinuation were tabulated and presented by diagnosis (Tables 1 and 2).

Descriptive statistics of the obtained values for selected outcome measures are presented by diagnosis and visit in graphic and tabulated form in the main body of the article or as Supplemental Material (available at www.ophthalmologyscience.org). Selected outcome measures include BCVA, LLVA, and LLD (Fig S1A−C, available at www.ophthalmologyscience.org), the 2 MP variables PRT and AT (Fig 1A, B), CCT (red, green, and blue, Fig 2A−C), rod intercept time (RIT) on DA testing (Fig 3), and matching descriptive statistics (Tables S1−S9, available at www.ophthalmologyscience.org).

To explore the selected VF assessments (BCVA, LLVA, CCT, MP, and DA) as potential suitable functional biomarkers to describe disease progression during eAMD and iAMD, we evaluated the data using box plots of actual values of the different VF variables (Figs 1−3, Fig S1, available at www.ophthalmologyscience.org) and different MMRM models generating predicted values for the statistical analysis (Tables 3−10). This allowed the complete data set to contribute to the analysis and ability to control for covariates that were found to significantly influence different functional variables. We applied MMRM models with predicted absolute values under the assumption of normal distribution (Tables 3−6 for BCVA, LLVA, LLD, MP PRT, MP AT, CCT red, CCT blue, CCT green, and RIT; and models evaluating the change from baseline (Tables 7−10). A mixed model with repeated measures (MMRM) was used to

Table 2. Reasons for Study Discontinuation

Reason for Study Discontinuation	Normal	Early AMD	Intermediate AMD
Study eye converted to neovascular AMD	0	0	3
Study eye converted to eAMD	1	0	0
Unrelated health problems	2	8	10
Death of spouse	1	0	0
Relocated	0	1	1
Lost to contact	0	1	1
Died	0	1	1
Total	**4**	**11**	**16**

AMD = age-related macular degeneration; eAMD = early age-related macular degeneration.

perform cross-sectional comparisons for these outcomes based on computed values by diagnosis at the 6-, 12-, 18-, and 24-month visits. The model was adjusted for baseline values of the outcome variable, visit, age, coronary artery disease (yes/no), dry eye (yes/no), and phakic status (yes/no). A diagnosis group-by-visit interaction term was included. Pairwise comparisons between diagnoses at each visit were based on predicted values from the model (least-square means) (Tables 3–6).

Descriptive statistics for change from baseline for the outcome measures were computed by diagnosis and visit and presented in tabular forms (Tables 7–10). An MMRM was used to analyze change from baseline. The longitudinal model was adjusted for visit, age, coronary artery disease, dry eye, and phakic status. A diagnosis group-by-visit interaction term was included. Pairwise comparisons between diagnoses at each visit were based on predicted values from the model (least-square means). Within-diagnosis group changes from baseline were also assessed on the basis of predicted values from the model (Tables 7–10).

During the analysis of the collected RIT data, it became apparent that the assumption of normal distribution of this data set was challenged by several aspects, including an observed ceiling effect at RIT = 20 minutes, which was entered in the data if reached as the DA test was stopped at this time interval. This prompted us to perform a second analysis of the RIT data using a data-driven approach to define the number of poor performers of RIT in each study group (controls, eAMD, and iAMD) relative to a "normal functional range" based on the age-matched control group at baseline by defining a lower limit of normal RIT as Mean RIT − 2 × standard deviation (SD) and the upper limit of the normal RIT at the mean RIT + 2 × SD (16.6 minutes; Table 11).

For all analyses, a P value < 0.05 was considered significant. No adjustment for multiple comparisons was applied, because in an exploratory study such as the current work the goal is to identify potential biomarkers of disease progression for future validation.

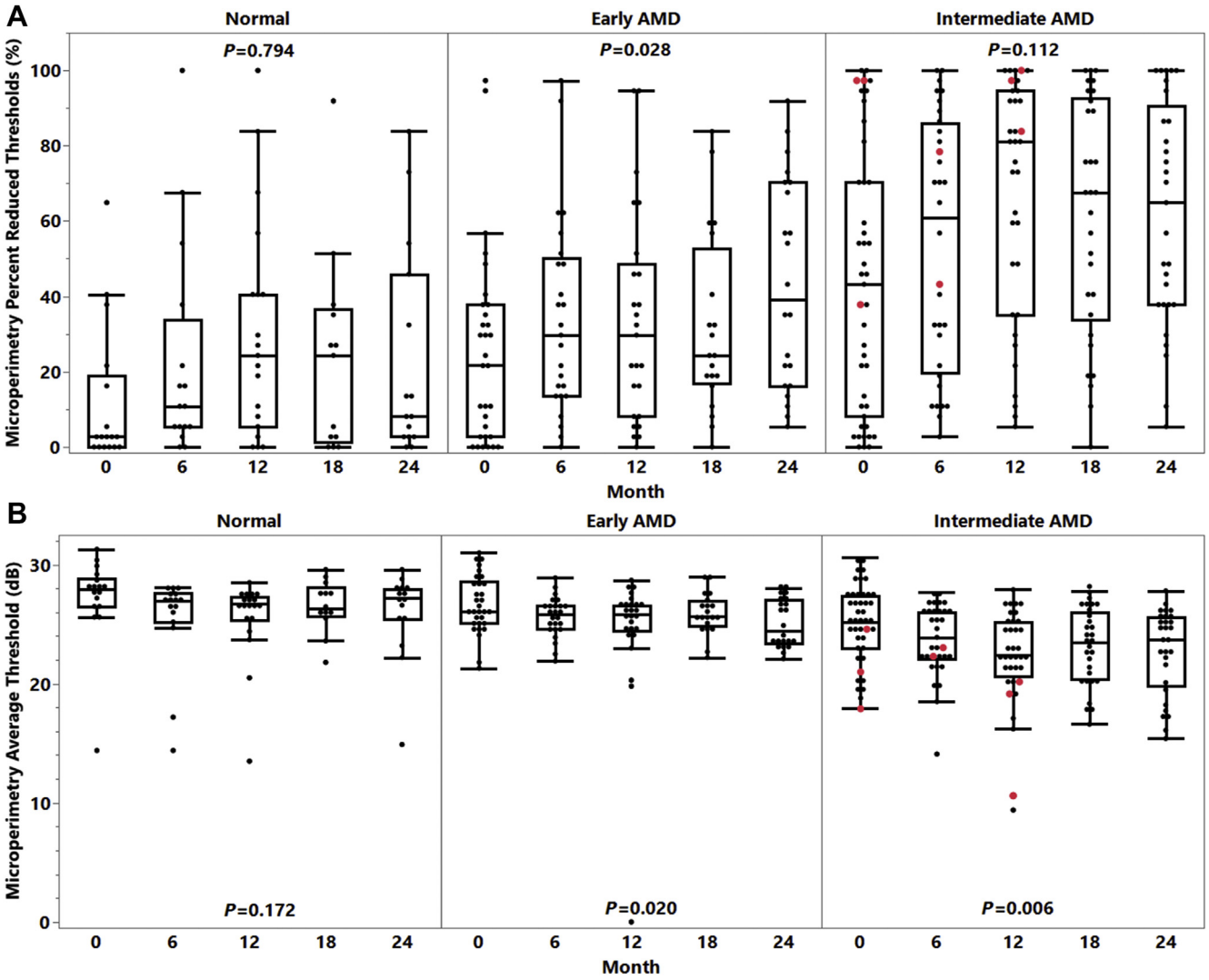

Figure 1. Longitudinal progression in microperimetry (MP) percent reduced threshold (PRT) (**A**) and absolute threshold (AT) (**B**) for the normal control, early age-related macular degeneration (eAMD), and intermediate AMD (iAMD) participants over 24 months. Patients with iAMD who converted to neovascular AMD are marked in red. Individual actual visual function (VF) data from all individuals who completed the respective VF assessments are shown by study time point. Box and whisker plots show 25th, 50th, and 75th percentiles. Lower whisker extends to the 25th percentile minus 1.5 times the interquartile range. Upper whisker extends to the 75th percentile plus 1.5 times the interquartile range. Outliers are points outside range of the whiskers. *P* values are model based and indicate significance of change from baseline to month 24 within each group.

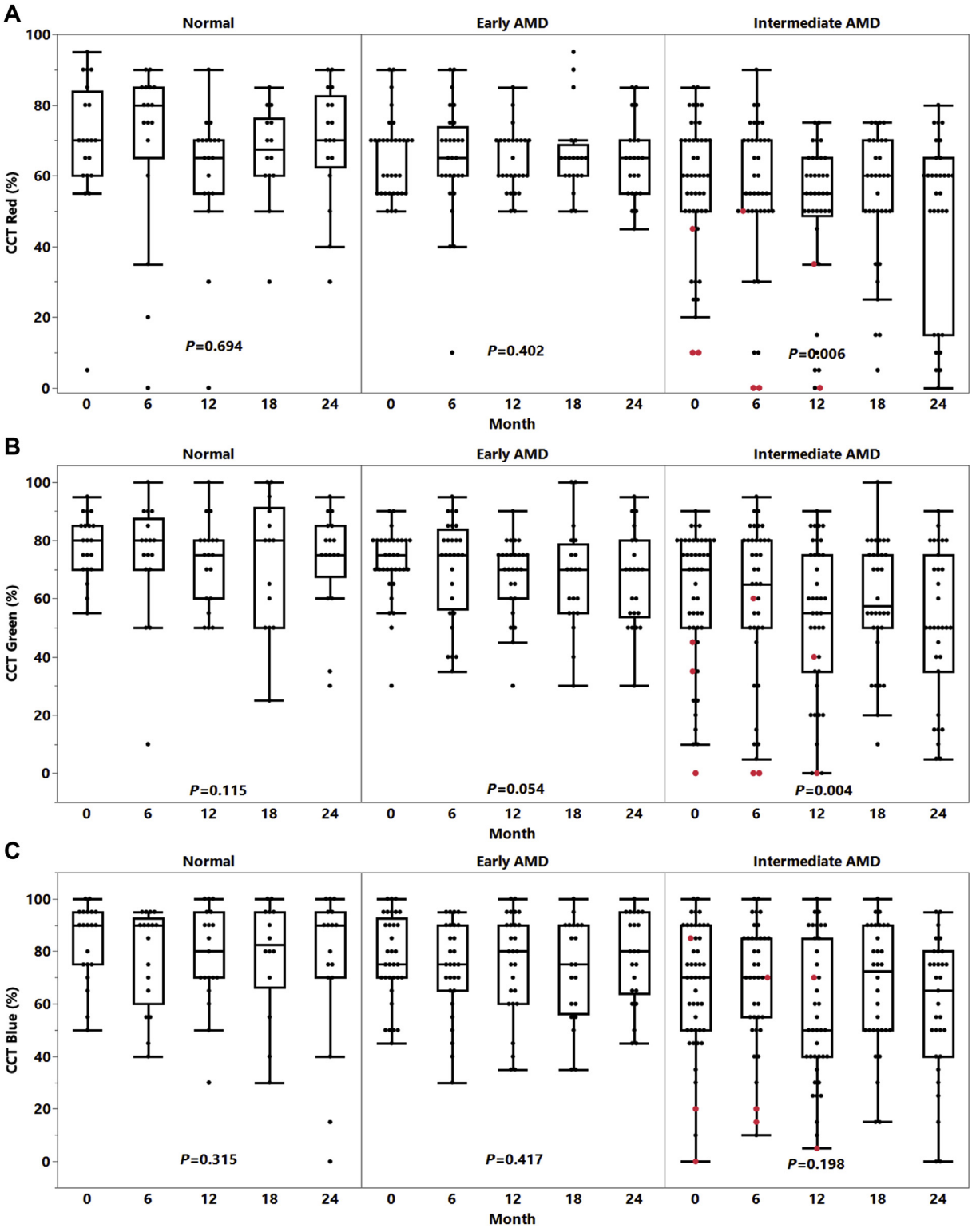

Figure 2. Longitudinal progression in cone contrast test (CCT) red (**A**), green (**B**), and blue (**C**) for the normal control, early age-related macular degeneration (AMD), and intermediate AMD (iAMD) subjects over 24 months, respectively. Patients with iAMD who converted to neovascular AMD are marked in red. For CCT red, green, and blue, 1 neovascular AMD participant did not have values at month 12. Individual actual visual function (VF) data from all individuals who completed the respective VF assessments are shown by study time point. Box and whisker plots show 25th, 50th, and 75th percentiles. Lower whisker extends to the 25th percentile minus 1.5 times the interquartile range. Upper whisker extends to the 75th percentile plus 1.5 times the interquartile range. Outliers are points outside range of the whiskers. *P* values are model based and indicate significance of change from baseline to month 24 within each group.

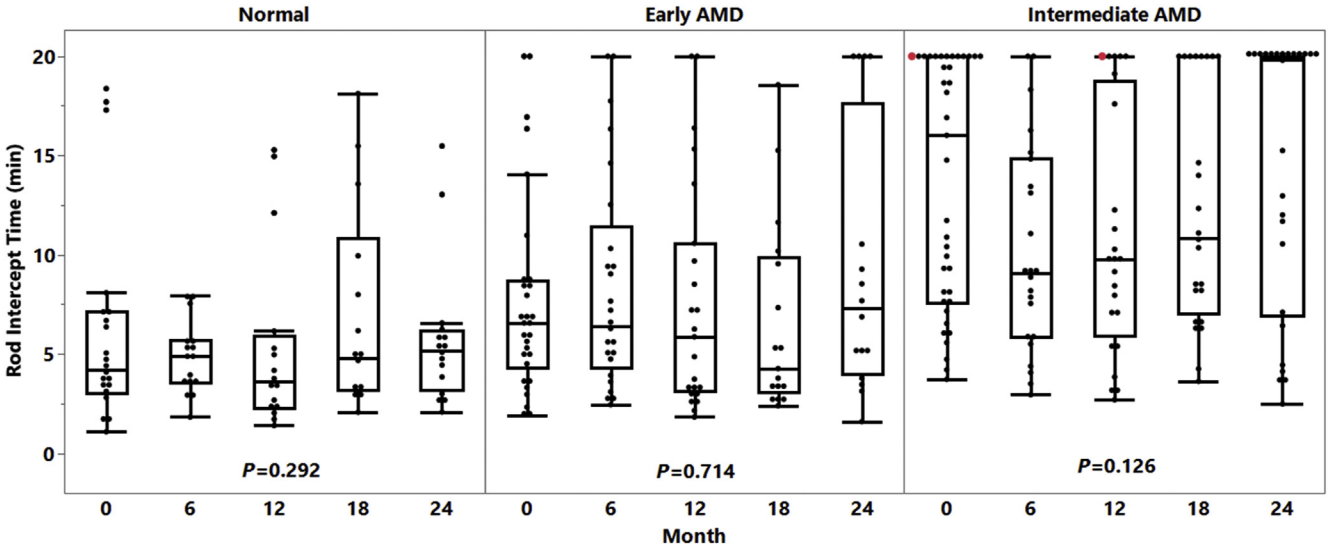

Figure 3. Longitudinal progression in dark adaptation (DA) rod intercept time (RIT) for the normal control, early age-related macular degeneration (AMD), and intermediate AMD (iAMD) subjects over 24 months. Patients with iAMD who converted to neovascular AMD are marked in red. Individual visual function (VF) data from all individuals who completed the respective VF assessments are shown by study time point. One converting patient did not have any values for rod intercept on DA. One patient did not have values and 6 and 12 months, and 1 patient did not have values at baseline and 6 months. Box and whisker plots show 25th, 50th, and 75th percentiles. Lower whisker extends to the 25th percentile minus 1.5 times the interquartile range. Upper whisker extends to the 75th percentile plus 1.5 times the interquartile range. Outliers are points outside range of the whiskers. *P* values are model based and indicate significance of change from baseline to month 24 within each group.

DNA Extraction, Genotyping, and Genetic Analysis

DNA extractions from 100 whole-blood samples from all consenting participants (20 normal, 33 eAMD, 47 iAMD) were performed using a silica adsorption-based method (MagNA Pure 96 DNA, Viral NA Small Volume Kit; Roche Applied Science). Normalized DNA concentration (60 ng/μl) was used for the respective TaqMan Assays (ThermoFisher Scientific) and run in duplicates on Fluidigm 192 × 24 genotyping arrays. The 9 analyzed single-nucleotide polymorphisms (SNPs) were selected on the basis of their association with AMD risk[29,30] (Table 12). In addition, each SNP was coded additively as the number of risk alleles in each SNP (0, 1, 2), and for burden analysis, a genetic-burden score was computed as the unweighted sum of risk alleles in all genotyped SNPs. Because all SNPs had minor allele frequencies $> 10\%$, the weighted and unweighted burden scores were highly correlated ($r = 0.93$, $P = 2e\text{-}16$); thus, for simplicity, only unweighted burden scores were reported.

A basic linear regression model controlling for covariates including baseline score, age, color assessment and diagnosis, dry eye, and phakic status was used to test the association between each genetic variant (each SNP and burden score) and selected VF test variables at 2 time points (12 and 24 months). The VF variables were actual values for low luminance vision (LLVA and LLD), microperimetry (AT and PRT), DA, and CCT (CCT_RED). In addition, an extended linear model including the SNP diagnosis interaction and testing for differential genetic effect by diagnosis group was performed. For the genetic analyses, we report the genetic effects based on uncorrected *P* values without multiple testing correction (as part of an exploratory analysis).

Results

From the initial 101 subjects (33 eAMD, 47 iAMD, and 21 controls) enrolled in a prospective, observational natural history study at Duke Eye Center, 70 completed the 24-month study visit (representing ∼15% dropout rate per year) (Table 1). The reasons for study discontinuation are presented in Table 2. Baseline demographics have been published and included covariate data potentially relevant in AMD.[20]

At the 12-month time point, 3 participants had progressed from iAMD to neovascular AMD, and 1 participant from the normal control group progressed to eAMD based on the AREDS classification.[2] Therefore, in the first year we observed a conversion rate of $3/47 = 6.4\%$. Although these participants were removed from the clinical study after conversion to a more advanced AMD stage, their individual VF data contribute to the model at baseline and 6 and 12 months, respectively. The data for the 3 converters to neovascular AMD are highlighted as red encircled data points in Figures 1 to 6 and in Figure S1 (available at www.ophthalmologyscience.org). At 24 months, none of the remaining participants showed signs of progression to a more severe disease stage based on the AREDS classification,[2] resulting in an overall progression rate per year of $3/87 = 3.5\%$ over the study period.

Cross-sectional Comparisons and Impact of Baseline Values as a Covariate

We first evaluated which of the VF variables were able to distinguish eAMD and iAMD disease stages from normal controls in cross-sectional comparisons at 6, 12, 18, and 24 months after controlling for covariates (baseline value, visit, age, coronary artery disease, dry eye and phakic status, and a group-by-visit interaction) (Tables 3–6). Furthermore, we explored the impact of baseline value as a covariate in the model by comparing the analysis of the MMRM models

Table 3. Cross-sectional Comparisons at the 6-, 12-, 18-, and 24-Month Visits for BCVA, LLVA, and LLD among Normal Control, Early AMD, and Intermediate AMD Groups

Test	Visit	Normal N Mean (SE)	Early N Mean (SE)	Intermediate N Mean (SE)	*P* Value Early vs. Normal	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
BCVA	6	15 85.59 (1.47)	30 83.90 (1.22)	40 84.12 (1.18)	0.295	0.339	0.856
	12	17 83.27 (1.42)	27 85.48 (1.30)	40 82.48 (1.17)	0.181	0.604	**0.022**
	18	15 83.01 (1.38)	20 85.98 (1.28)	32 84.27 (1.18)	0.066	0.390	0.200
	24	16 81.15 (1.45)	22 84.47 (1.34)	31 82.94 (1.25)	0.058	0.271	0.300
LLVA	6	15 70.72 (1.99)	30 71.06 (1.64)	40 70.60 (1.59)	0.876	0.958	0.790
	12	17 72.35 (1.73)	27 70.95 (1.59)	40 68.24 (1.47)	0.473	**0.028**	0.083
	18	15 69.86 (1.74)	20 71.55 (1.61)	32 70.10 (1.50)	0.398	0.895	0.386
	24	16 68.39 (1.81)	22 70.09 (1.67)	31 71.10 (1.60)	0.427	0.184	0.580
LLD	6	15 13.86 (2.09)	30 11.91 (1.61)	40 13.75 (1.50)	0.418	0.960	0.324
	12	17 9.96 (1.33)	27 13.37 (1.26)	40 14.42 (1.15)	**0.024**	**0.002**	0.381
	18	15 12.12 (1.36)	20 13.44 (1.28)	32 14.37 (1.18)	0.383	0.111	0.468
	24	16 11.85 (1.66)	22 13.29 (1.52)	31 11.80 (1.41)	0.473	0.978	0.387

AMD = age-related macular degeneration; BCVA = best-corrected visual acuity; LLD = low-luminance deficit; LLVA = low-luminance visual acuity; SE = standard error.
Data are units of BCVA; LLVA and LLD are ETDRS letters. Data are predicted values from a mixed model with repeated measures, controlling for visit, baseline values, age, coronary artery disease, dry eye, and cataract with group-by-visit interaction term. Pairwise comparisons are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

using the predicted absolute values with baseline correction (Tables 3–6) with those of change from baseline (Tables 7–10).

At 24 months, the iAMD group and control group significantly differed on the following VF tests: MP PRT ($P = 0.004$, Table 4), CCT red ($P = 0.011$, Table 5), and

Table 4. Cross-sectional Comparisons at the 6-, 12-, 18-, and 24-Month Visits for Microperimetry PRT and AT among Normal Control, Early AMD, and Intermediate AMD Groups

Test	Visit	Normal N Mean (SE)	Early N Mean (SE)	Intermediate N Mean (SE)	*P* Value Early vs. Normal	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
PRT	6	12 32.11 (8.48)	24 30.99 (6.60)	29 42.16 (6.49)	0.900	0.259	0.116
	12	13 33.43 (8.31)	24 29.88 (6.83)	32 56.01 (6.46)	0.694	**0.012**	**<0.001**
	18	10 23.31 (8.73)	19 31.75 (6.80)	26 56.50 (6.61)	0.365	**0.001**	**0.001**
	24	11 23.05 (8.51)	21 44.25 (6.78)	25 51.34 (6.89)	**0.026**	**0.004**	0.363
AT	6	12 24.30 (0.82)	24 25.44 (0.66)	30 24.51 (0.64)	0.162	0.788	0.152
	12	13 24.98 (1.18)	25 24.41 (0.92)	33 23.31 (0.84)	0.676	0.201	0.297
	18	10 25.80 (0.84)	19 25.49 (0.68)	26 23.53 (0.66)	0.718	**0.008**	**0.005**
	24	11 25.40 (0.92)	21 24.68 (0.74)	25 23.63 (0.73)	0.473	0.077	0.197

AMD = age-related macular degeneration; AT = average threshold; PRT = percent reduced threshold; SE = standard error. Data are predicted values from a mixed model with repeated measures, controlling for visit, baseline values, age, coronary artery disease, dry eye, and cataract with group-by-visit interaction term. Pairwise comparisons are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

Table 5. Cross-sectional Comparisons at the 6-, 12-, 18-, and 24-Month Visits for CCTs of Red, Green, and Blue Cones among Normal Control, Early AMD, and Intermediate AMD Groups

Test	Visit	Normal N Mean (SE)	Early N Mean (SE)	Intermediate N Mean (SE)	*P* Value Early vs. Normal	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
CCT Red (%)	6	14 71.43 (3.87)	28 61.05 (3.18)	39 59.80 (3.11)	**0.013**	**0.005**	0.692
	12	16 60.46 (3.67)	27 60.33 (3.34)	38 52.72 (3.07)	0.974	**0.052**	**0.021**
	18	13 62.72 (4.05)	20 62.46 (3.51)	32 54.69 (3.25)	0.955	0.070	**0.038**
	24	15 63.91 (4.27)	22 60.45 (3.75)	31 51.26 (3.52)	0.492	**0.011**	**0.030**
CCT Green (%)	6	14 67.38 (4.05)	28 66.17 (3.37)	39 64.94 (3.34)	0.787	0.582	0.722
	12	16 63.24 (3.66)	27 63.20 (3.37)	39 58.04 (3.20)	0.992	0.201	0.116
	18	13 64.08 (4.16)	20 61.35 (3.67)	32 62.16 (3.42)	0.567	0.676	0.833
	24	15 62.85 (4.21)	22 60.72 (3.76)	31 55.74 (3.61)	0.669	0.148	0.233
CCT Blue (%)	6	14 77.13 (4.12)	28 76.24 (3.56)	39 75.65 (3.56)	0.839	0.729	0.859
	12	16 75.46 (4.22)	27 77.54 (3.93)	39 64.29 (3.67)	0.667	**0.018**	**0.001**
	18	13 77.60 (4.79)	20 74.35 (4.23)	32 71.43 (3.92)	0.558	0.245	0.509
	24	15 69.97 (5.58)	22 77.49 (4.87)	31 64.62 (4.53)	0.269	0.412	**0.023**

AMD = age-related macular degeneration; CCT = cone contrast test; SE = standard error. Data are predicted values from a mixed model with repeated measures, controlling for visit, baseline values, age, coronary artery disease, dry eye, and cataract with group-by-visit interaction term. Pairwise comparisons are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

DA RIT ($P = 0.007$, Table 6). Likewise, iAMD and eAMD patients significantly differed for CCT red ($P = 0.030$, Table 5), CCT blue ($P = 0.023$, Table 5), and DA RIT ($P = 0.027$, Table 6), whereas MP PRT ($P = 0.026$, Table 4) distinguished eAMD from normal. The BCVA, LLVA, and LLD showed no significant changes between study groups at 24 months (Table 3).

When considering earlier time points at which significant differences were detected, CCT red ($P < 0.05$ at 6, 12, and 24 months, Table 5) and MP PRT ($P < 0.05$ at 12, 18, and 24 months, Table 4) were most consistently able to distinguish patients with iAMD significantly from normal patients over consecutive visits. In these analyses, however, LLVA ($P = 0.028$, Table 3) and LLD ($P = 0.002$, Table 3) only distinguished patients with iAMD from normal controls at 12 months but did not show differences at later time points, independent of the MMRM models used for LLVA or LLD, either based on predicted actual values (Table 3) or on change from baseline (Table 7). For BCVA, it appears that the eAMD group experienced deficits compared with normal controls at 18 and 24 months (Table 7). However, this appeared to

Table 6. Cross-sectional Comparisons at the 6-, 12-, 18-, and 24-Month Visits for Rod Intercept Time on Dark Adaptation Testing among Normal Control, Early AMD, and Intermediate AMD Groups

Visit	Normal N Mean (SE)	Early N Mean (SE)	Intermediate N Mean (SE)	*P* Value Early vs. Normal	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
6	14 8.79 (1.14)	23 10.16 (0.99)	22 10.68 (1.14)	0.230	0.126	0.615
12	14 9.15 (1.31)	21 8.94 (1.23)	23 9.59 (1.27)	0.890	0.781	0.633
18	14 10.72 (1.27)	16 8.60 (1.21)	21 11.17 (1.23)	0.159	0.764	0.062
24	15 9.54 (1.33)	14 10.55 (1.35)	24 13.97 (1.26)	0.545	**0.007**	**0.027**

AMD = age-related macular degeneration; SE = standard error. Data are predicted values from a mixed model with repeated measures, controlling for visit, baseline values, age coronary artery disease, dry eye, and phakic status with group-by-visit interaction term. Pairwise comparisons are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

Table 7. Change from Baseline Comparisons at the 6-, 12-, 18-, and 24-Month Visits for BCVA, LLVA, and LLD within and among Normal Control, Early AMD, and Intermediate AMD Groups

Test	Visit	Normal N Mean (SE) (*P* Value)	Early N Mean (SE) (*P* Value)	Intermediate N Mean (SE) (*P* Value)	*P* Value Normal vs. Early	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
BCVA	6	12 3.22 (1.62) (**0.049**)	24 2.44 (1.34) (0.070)	30 2.69 (1.28) (**0.037**)	0.665	0.755	0.860
	12	13 0.91 (1.53) (0.555)	25 3.97 (1.39) (**0.005**)	33 0.98 (1.26) (0.435)	0.086	0.964	**0.035**
	18	10 0.68 (1.43) (0.634)	19 4.32 (1.33) (**0.001**)	26 2.57 (1.23) (0.039)	**0.029**	0.212	0.200
	24	11 −1.16 (1.44) (0.423)	21 2.72 (1.35) (**0.046**)	25 1.14 (1.28) (0.375)	**0.024**	0.148	0.272
LLVA	6	15 0.36 (2.00) (0.857)	30 1.24 (1.67) (0.459)	40 1.47 (1.60) (0.358)	0.692	0.599	0.894
	12	17 2.02 (1.79) (0.260)	27 1.11 (1.65) (0.503)	40 −0.87 (1.51) (0.563)	0.651	0.125	0.214
	18	15 −0.44 (1.83) (0.809)	20 1.61 (1.69) (0.342)	32 0.84 (1.56) (0.592)	0.331	0.509	0.657
	24	16 −1.87 (1.86) (0.319)	22 0.12 (1.73) (0.945)	31 1.73 (1.64) (0.295)	0.366	0.085	0.391
LLD	6	15 2.52 (2.24) (0.262)	30 1.05 (1.78) (0.557)	40 1.08 (1.68) (0.522)	0.562	0.551	0.987
	12	17 −1.36 (1.80) (0.449)	27 2.53 (1.64) (0.127)	40 1.68 (1.49) (0.261)	0.060	0.111	0.603
	18	15 0.81 (1.54) (0.597)	20 2.44 (1.45) (0.096)	32 1.80 (1.38) (0.195)	0.324	0.513	0.639
	24	16 0.47 (1.76) (0.789)	22 2.33 (1.64) (0.160)	31 −0.69 (1.57) (0.662)	0.369	0.551	0.091

AMD = age-related macular degeneration; BCVA = best-corrected visual acuity; LLD = low-luminance deficit; LLVA = low-luminance visual acuity; SE = standard error.
Data are units of BCVA, LLVA, and LLD are ETDRS letters. Data are predicted change from baseline values from a mixed model with repeated measures, controlling for visit, age, coronary artery disease, dry eye, and phakic status with group-by-visit interaction term. *P* values shown in brackets for each diagnosis at each visit assess within group changes from baseline. Pairwise comparisons between groups are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

be related to the BCVA status at baseline, because normal and eAMD did not differ in the MMRM model using predicted absolute values (Table 3).

All iAMD subjects at baseline and all subsequent study time points showed worse RIT than the normal age-matched controls (Fig 3, Table S9, available at www.ophthalmologyscience.org) and generally had significantly worse mean RIT values than the eAMD subjects at the different study time points apart from month 6 (Fig 3 and Table S9, available at www.ophthalmologyscience.org, $P < 0.05$). The finding of the preexisting deficit in RIT at baseline that we previously reported[20] led to the inclusion of the RIT baseline value as a covariate in our MMRM models of predicted actual values (Table 6) to evaluate the influence of RIT deficit for the follow-up time points. We found that most of the significant differences among the iAMD, control, and eAMD groups at the different follow-up study time points disappeared in this analysis, with the exception of a significant difference remaining between the iAMD group and the normal group and eAMD group at month 24 (Table 6, $P < 0.05$).

Overall, these cross-sectional comparisons with the different MMRM models demonstrated that MP PRT, CCT red, and DA RIT revealed a difference among iAMD, eAMD, and normal participants most consistently at various visits over the period of 24 months, whereas RIT longitudinal analysis displayed a strong influence of a preexisting dark adaption deficit at baseline.

Longitudinal within Group Comparisons

To perform longitudinal analyses within each group, we used MMRM models to evaluate the change from baseline with covariate corrections for visit, age, coronary artery disease, dry eye, phakic status, and a group-by-visit interaction term (Tables 7−10).

Longitudinal analyses of VF tests were conducted within the control, eAMD, and iAMD groups to explore the VF change over the period of 24 months relative to baseline and to explore functional progression at earlier time points (6, 12, and 18 months, Tables 7−10).

Although generally only subtle longitudinal changes relative to baseline were observed within each group over 24 months, both MP measures (PRT and AT), CCT red, and CCT green revealed significant longitudinal deterioration relative to baseline. The CCT blue, LLVA, and LLD, however, did not show significant changes in any longitudinal analyses from baseline to 24 months.

In iAMD subjects, the longitudinal VF changes from baseline became apparent at 12 months and were found to be significant ($P < 0.05$) for MP PRT at 12 and 18 months (Table 8, Fig 1A), MP AT at 12, 18, and 24 months

Table 8. Change from Baseline Comparisons at the 6-, 12-, 18-, and 24-Month Visits for Microperimetry PRT and AT within and among Normal Control, Early AMD, and Intermediate AMD Groups

Test	Visit	Normal N Mean (SE) (*P* Value)	Early N Mean (SE) (*P* Value)	Intermediate N Mean (SE) (*P* Value)	*P* Value Normal vs. Early	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
PRT	6	12 11.54 (9.10) (0.208)	24 4.21 (7.37) (0.570)	29 5.33 (7.29) (0.467)	0.428	0.485	0.875
	12	13 12.83 (9.56) (0.183)	24 3.04 (8.06) (0.707)	32 19.06 (7.62) (**0.014**)	0.344	0.522	0.047
	18	10 3.02 (10.40) (0.772)	19 5.22 (8.19) (0.526)	26 20.09 (7.96) (**0.013**)	0.845	0.115	0.091
	24	11 2.67 (10.20) (0.794)	21 18.53 (8.25) (**0.028**)	25 13.27 (8.29) (0.112)	0.166	0.342	0.564
AT	6	12 −2.51 (0.91) (**0.007**)	24 −1.17 (0.74) (0.117)	30 −1.37 (0.72) (0.060)	0.142	0.192	0.773
	12	13 −1.81 (1.22) (0.142)	25 −2.18 (0.97) (**0.027**)	33 −2.49 (0.89) (**0.006**)	0.788	0.605	0.774
	18	10 −1.19 (0.94) (0.212)	19 −1.16 (0.77) (0.134)	26 −2.41 (0.75) (**0.002**)	0.975	0.192	0.102
	24	11 −1.42 (1.03) (0.172)	21 −1.97 (0.83) (**0.020**)	25 −2.28 (0.82) (**0.006**)	0.625	0.436	0.730

AMD = age-related macular degeneration; AT = average threshold; PRT = percent reduced threshold; SE = standard error. Data are predicted change from baseline values from a mixed model with repeated measures, controlling for visit, age, coronary artery disease, dry eye, and phakic status with group-by-visit interaction term. *P* values shown in brackets for each diagnosis at each visit assess within group changes from baseline. Pairwise comparisons between groups are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

(Table 8, Fig 1B), CCT red at 12, 18, and 24 months (Table 9, Fig 2A), and CCT green at 12 and 24 months (Table 9, Fig 2B).

Subjects with eAMD also showed significant worsening relative to their baseline in several functional tests at different visits, including BCVA (Table 7, Fig S1A,

Table 9. Change from Baseline Comparisons at the 6-, 12-, 18-, and 24-Month Visits for Cone Contrast Tests of Red, Green, and Blue Cones within and among Normal Control, Early AMD, and Intermediate AMD Groups

Test	Visit	Normal N Mean (SE) (*P* Value)	Early N Mean (SE) (*P* Value)	Intermediate N Mean (SE) (*P* Value)	*P* Value Normal vs. Early	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
CCT Red (%)	6	14 5.97 (3.90) (0.129)	28 −2.43 (3.30) (0.462)	39 −1.22 (3.19) (0.704)	**0.047**	0.071	0.702
	12	16 −5.01 (3.74) (0.184)	27 −3.33 (3.48) (0.341)	38 −8.47 (3.17) (**0.009**)	0.690	0.378	0.121
	18	13 −2.86 (4.18) (0.495)	20 −1.31 (3.68) (0.722)	32 −6.66 (3.39) (**0.051**)	0.748	0.395	0.165
	24	15 −1.68 (4.26) (0.694)	22 −3.21 (3.82) (0.402)	31 −9.88 (3.57) (**0.006**)	0.762	0.088	0.110
CCT Green (%)	6	14 −2.19 (4.05) (0.590)	28 −1.94 (3.43) (0.573)	39 −1.21 (3.28) (0.712)	0.955	0.816	0.830
	12	16 −6.35 (3.68) (0.088)	27 −4.93 (3.44) (0.155)	39 −8.08 (3.14) (**0.011**)	0.732	0.650	0.325
	18	13 −5.52 (4.16) (0.188)	20 −6.87 (3.72) (0.068)	32 −4.14 (3.38) (0.223)	0.777	0.753	0.472
	24	15 −6.77 (4.25) (0.115)	22 −7.49 (3.84) (**0.054**)	31 −10.5 (3.59) (**0.004**)	0.887	0.438	0.475
CCT Blue (%)	6	14 1.27 (4.41) (0.773)	28 3.08 (3.82) (0.421)	39 4.98 (3.67) (0.178)	0.701	0.404	0.596
	12	16 −0.35 (4.32) (0.936)	27 4.28 (4.08) (0.297)	39 −6.30 (3.68) (0.089)	0.345	0.189	**0.006**
	18	13 1.97 (4.97) (0.692)	20 1.02 (4.40) (0.817)	32 0.78 (3.98) (0.844)	0.868	0.822	0.958
	24	15 −5.85 (5.78) (0.315)	22 4.11 (5.05) (0.417)	31 −5.95 (4.60) (0.198)	0.157	0.987	0.080

AMD = age-related macular degeneration; CCT = cone contrast test; SE = standard error. Data are predicted change from baseline values from a mixed model with repeated measures, controlling for visit, age, coronary artery disease, dry eye, and phakic status with group-by-visit interaction term. *P* values shown in brackets for each diagnosis at each visit assess within group changes from baseline. Pairwise comparisons between groups are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

Table 10. Change from Baseline Comparisons at the 6-, 12-, 18-, and 24-Month Visits for Rod Intercept on Dark Adaptation, within and among Normal Control, Early AMD, and Intermediate AMD Groups

Visit	Normal N Mean (SE) (*P* Value)	Early N Mean (SE) (*P* Value)	Intermediate N Mean (SE) (*P* Value)	*P* Value Normal vs. Early	*P* Value Intermediate vs. Normal	*P* Value Intermediate vs. Early
6	14 0.78 (1.47) (0.596)	24 0.68 (1.30) (0.601)	23 −0.05 (1.35) (0.972)	0.949	0.584	0.575
12	17 0.14 (1.46) (0.922)	24 −0.48 (1.43) (0.737)	28 −2.58 (1.37) (0.063)	0.708	0.092	0.144
18	15 2.49 (1.59) (0.121)	17 −0.87 (1.50) (0.563)	25 −1.26 (1.41) (0.376)	0.074	**0.033**	0.807
24	15 1.59 (1.50) (0.292)	17 0.53 (1.44) (0.714)	26 2.10 (1.36) (0.126)	0.541	0.754	0.300

AMD = age-related macular degeneration; SE = standard error. Data are predicted change from baseline values from a mixed model with repeated measures, controlling for visit, age, coronary artery disease, dry eye, and phakic status with group-by-visit interaction term. *P* values shown in brackets for each diagnosis at each visit assess within group changes from baseline. Pairwise comparisons between groups are based on least-squares means. Bold values denote statistical significance ($P < 0.05$).

available at www.ophthalmologyscience.org), MP PRT (Table 8, Fig 1A), MP AT (Table 8, Fig 1B), and CCT green (Table 9, Fig 2B). Notably, VF deterioration in the eAMD group relative to baseline was significant for BCVA at 12, 18, and 24 months, for MP AT at 12 and 24 months, and for MP PRT and CCT green at 24 months.

Within normal subjects, the only VF impairment relative to baseline was observed in BCVA (Table 7, Fig S1A, available at www.ophthalmologyscience.org) and MP AT at 6 months (Table 8, Fig 1B), whereas VF in normal participants was stable at all visits and for all psychophysical measures during the study period of 24 months.

While exploring the MMRM models for any longitudinal change in RIT on DA within the iAMD group or eAMD group, no significant change over time was observed under the assumption of normal distribution of the RIT data (Table 10).

Because the RIT data collected were limited by a ceiling effect at RIT = 20 minutes at which the DA test was stopped, we explored a categorical analysis aimed to identify subjects with poor performance in DA in all 3 study groups at all study time points. "Poor performance" was defined as an RIT value larger than the upper limit (Mean + 2 SD = 16.6 minutes) of the age-matched control group at baseline (Table 11). A higher proportion of iAMD subjects (48.7%) struggled with worse DA performance compared with the normal group (15.8%) at baseline, which confirmed results from our previous work.[20] Furthermore, an important observation was that particular iAMD subjects who had a poor performance at baseline were less likely to attempt a subsequent examination, leading to a preferential loss of data from the poor performers from our study data between baseline and 6 months in this group. Table 11 demonstrates that 19 iAMD subjects (~48.7%) had poor performance at baseline versus only 3

Table 11. Classification of Dark Adaptation Data into Poor Performers versus Normal Performers Based on the RIT

	Normal Frequency (%)			Early AMD Frequency (%)			Intermediate AMD Frequency (%)		
Visit	Normal Performance RIT ≤16.6*	*Bad* Performance RIT >16.6	*N*	Normal Performance RIT ≤16.6	*Bad* Performance RIT >16.6	*N*	Normal Performance RIT ≤16.6	*Bad* Performance RIT >16.6	*N*
0	16 (84.21)	3 (15.79)	19	26 (89.66)	3 (10.34)	29	20 (51.28)	19 (48.72)	39
6	14 (100.00)	0	14	21 (91.30)	2 (8.70)	23	19 (86.36)	3 (13.64)	22
12	14 (100.00)	0	14	20 (95.24)	1 (4.76)	21	17 (73.91)	6 (26.09)	23
18	13 (92.86)	1 (7.14)	14	15 (93.75)	1 (6.25)	16	16 (76.19)	5 (23.81)	21
24	15 (100.00)	0	15	12 (85.71)	2 (14.29)	14	12 (50.00)	12 (50.00)	24
Total	72	4	76	94	9	103	84	45	129

AMD = age-related macular degeneration; RIT = rod intercept time.
Poor performance was defined to reside outside of the upper limit (Mean RIT + 2 × SD) of a "normal functional range" of the age-matched control group at baseline. Absolute frequency and (%) of the total group is given for all assessments at respective visits for normal, eAMD, and iAMD groups. This data analysis only includes observations that have complete information for the covariates (baseline value, age, coronary artery disease, dry eye, and phakic status) to make the analysis comparable with the RIT analysis presented in Table 6.
Mean + 2 SDs = 16.6 min.
*RIT Mean (standard deviation [SD]) of normal at baseline = 6.20 (5.20).

Table 12. Nine Selected Single-Nucleotide Polymorphisms Included for Genotyping in this Study that Have Been Associated with Risk of AMD Development or AMD Progression

Gene	rsID	Minor Allele	Major Allele	Risk Allele	Reported Association with AMD	Observed MAF
APOE	rs429358	C	T	T (major)	Risk	0.15
ARMS2-HTRA1	rs2284665	T	G	T (minor)	Risk/Progression	0.37
ATF7IP2	rs12930861	G	C	G (minor)	Progression	0.11
C2-CFB-SKIV2L	rs429608	A	G	G (major)	Risk/Progression	0.1
C3	rs2230199	C	G	C (minor)	Risk/Progression	0.26
CFH	rs10922109	A	C	C (major)	Risk/Progression	0.32
CFH	rs570618	T	G	T (minor)	Risk	0.48
MMP9	rs1888235	T	C	C (major)	Progression	0.14
TNR	rs1894596	C	T	C (minor)	Progression	0.33

AMD = age-related macular degeneration; MAF = minor allele frequencies at least 0.1.

iAMD participants at 6 months. On the population level, this phenomenon resulted in an apparent, nonsignificant numerical improvement of the RIT, which reflects the preferential loss of poor performers from this study group (Table 10). Most important, after the 6-month time point, the number of subjects attempting the RIT assessment remained relatively stable, but nevertheless, the frequency of the poor performers increased over the next 18 months from 3 (∼13.6%) at 6 months to 12 (∼50%) at 24 months among the remaining iAMD subjects, whereas the frequency of poor performers over the same time period remained low and somewhat stable with 0% to 15% poor performers in the normal group and approximately 10% to 15% in the eAMD group.

Overall, subtle longitudinal VF deterioration in iAMD was detected by MP (PRT and AT), CCT red, and DA RIT testing, with the most prominent deterioration on the group level occurring between the 6- and 12-month visits, after which VF decline appeared to slow down or stabilize in the remaining trial population (Tables 7–10, Figs 1–3, Fig S1, available at www.ophthalmologyscience.org).

Genetic Analysis

To understand whether any of the candidate VF variables (LLVA, LLD, MP PRT, MP AT, CCT red, DA RIT) at 12 or 24 months are influenced by genetic risk factors known to be associated with onset or progression of AMD, we genotyped 9 selected SNPs in the 100 consenting of 101 trial participants (Table 12). This analysis revealed 4 suggestive associations between VF variables and specific SNPs (Table 13). The association with 1 SNP in Tenascin R and LLD and LLVA at 24 months remained significant even after correcting for multiple testing (critical $P < 0.001$).

In addition, we evaluated the association between the genetic burden score, defined as the sum of the risk alleles, with the disease stage (normal), early, intermediate, and the selected VF variables. The genetic burden score values ranged from 4 to 14 and correlated significantly with the disease stage (Spearman $r = 0.35$, $P = 0.0003$, Fig 5). Furthermore, the risk of belonging to a specific disease stage group was evaluated over the range of genetic burden scores using a multinominal logistic regression model that tested the association between the nominal outcome variable groups (normal, early, intermediate) and the respective genetic scores. This analysis revealed that the relative risk for having iAMD is 1.64 ($P = 0.0016$), meaning that with each risk allele, the relative risk for being in the iAMD group versus the normal group increases by 64%, whereas the relative risk for having eAMD is 1.13 (not significant).

Next, we evaluated whether the genetic burden score was associated with any of the selected VF variables at baseline by Spearman rank correlation analysis. We observed that the genetic burden moderately correlated with the dark adaption rod intercept score ($r = 0.42$, $P = 0.00005$). However, only a modest and nonsignificant correlation of the genetic burden with the 2 MP-based VF variables was observed (MP AT: $r = -0.19$, $P = 0.07$ and MP PRT: $r = 0.17$, $P = 0.11$). For CCT red, LLVA, and LLD, even lower associations were observed.

Association of Reticular Pseudodrusen and Hyperreflective Foci with VF Performance

Because the presence of RPD and HRFs has been reported to influence AMD progression to geographic atrophy,[31,32] we also evaluated whether these imaging biomarkers on SD-OCT at baseline were associated with AMD disease stage and with any VF deficit at baseline and 12 and 24 months. In this analysis, diagnoses were coded as an ordinal variable (normal = 1; early = 2; intermediate = 3), and a Spearman rank correlation analysis was performed. This investigation revealed that the presence of RPD and HRFs was significantly correlated with disease stage ($r = 0.56$ and 0.44, respectively, both $P < 0.000001$). Moreover, the proportion of study eyes positive for RPD or HRF was also associated with disease stage at baseline (Table 14, chi-square test, both $P < 0.00001$). In normal control eyes, the 2 features were not observed, whereas RPD in eAMD has been seen in only 4 of 33 eyes (12%) and in iAMD in 28 of 47 eyes (60%). Hyperreflective foci have been observed in 3 of 33 (9%) eAMD eyes and in 20 of 47 (43%) eyes in the iAMD group at baseline.

Among the VF variables, in particular RIT on DA at baseline ($r = 0.49$, $P < 0.000001$), 12 months ($r = 0.46$, $P = 0.0001$), and 24 months ($r = 0.44$, $P = 0.0006$) had a moderate association with the presence of RPD at baseline

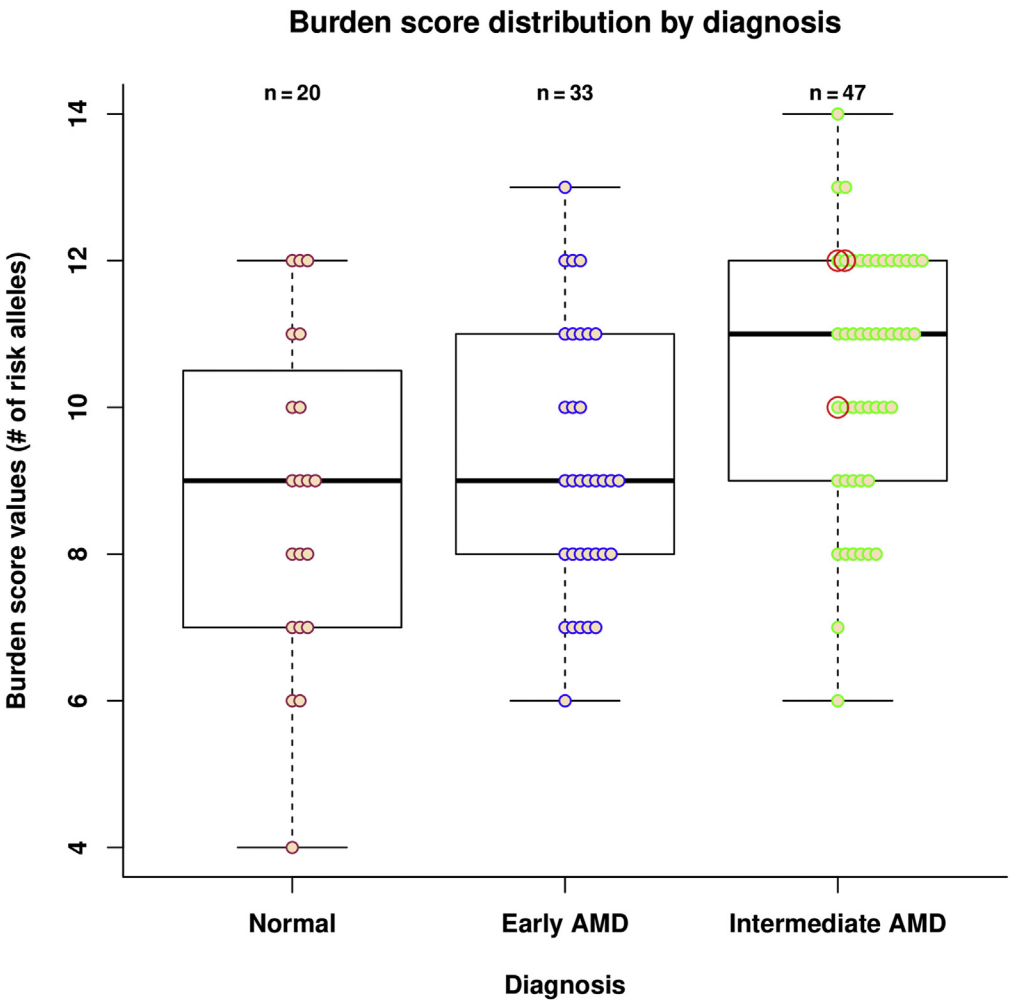

Figure 4. Association of genetic burden score with disease stage by Age-Related Eye Disease Study (AREDS) stage (Spearman $r = 0.35$, $P = 0.0003$). In this analysis, the diagnosis was treated as an ordinal variable according to the AREDS stage 1 (normal, n = 20), 2 (early age-related macular degeneration [AMD], n = 33), and 3 (intermediate AMD, n = 47). The 3 intermediate AMD patients who converted to neovascular AMD are highlighted by **red circles** and revealed values at the high end of the burden scores (10, 12, and 12, respectively). One subject did not consent to use of genetic material for genetic analysis (total n = 100 instead of 101).

(Fig 5A). The presence of RPD was not correlated with age ($r = -0.072$). However, if a correction for age is applied, the correlations remain significant and slightly increase for all visits ($r = 0.50$ at baseline, $r = 0.47$ at 12 months, and $r = 0.47$ at 24 months). Thus, the significant correlations between RIT and presence of RPD are unaffected by age in this study. The MP variables PRT and AT as well as CCT red demonstrated a weak to moderate association with this imaging biomarker at baseline (Fig 5B–D). These correlations at baseline remained significant ($P < 0.05$) at 12 months and 24 months, respectively ($r \geq 0.22$ for all correlations).

Likewise, the presence of HRFs at baseline showed a weak to moderate correlation with increased rod intercept in DA at baseline ($r = 0.31$, $P = 0.004$; Fig 6A), which was maintained at 12 months ($r = 0.44$, $P = 0.0003$) and 24 months ($r = 0.41$, $P = 0.001$). Furthermore, HRFs at baseline also displayed weak associations with MP PRT, MP AT, and CCT red at baseline (Fig 6B–D), which remained significant ($P < 0.05$) at 12 months and 24 months ($r \geq 0.29$).

Discussion

Currently, there is a paucity of treatments for patients with nonexudative AMD, and clinical trials for therapies in process are hampered by a lack of sensitive and reliable end points. To address this unmet need, the main objective of our study was to uncover sensitive functional outcomes for measuring early-to-intermediate AMD progression. In this study, we evaluated eAMD, iAMD, and age-matched control subjects using a variety of psychophysical tests including BCVA, LLVA, CCT, MP, and DA to detect early signs of AMD and disease progression over 24 months. In addition, we assessed whether a limited number of genetic

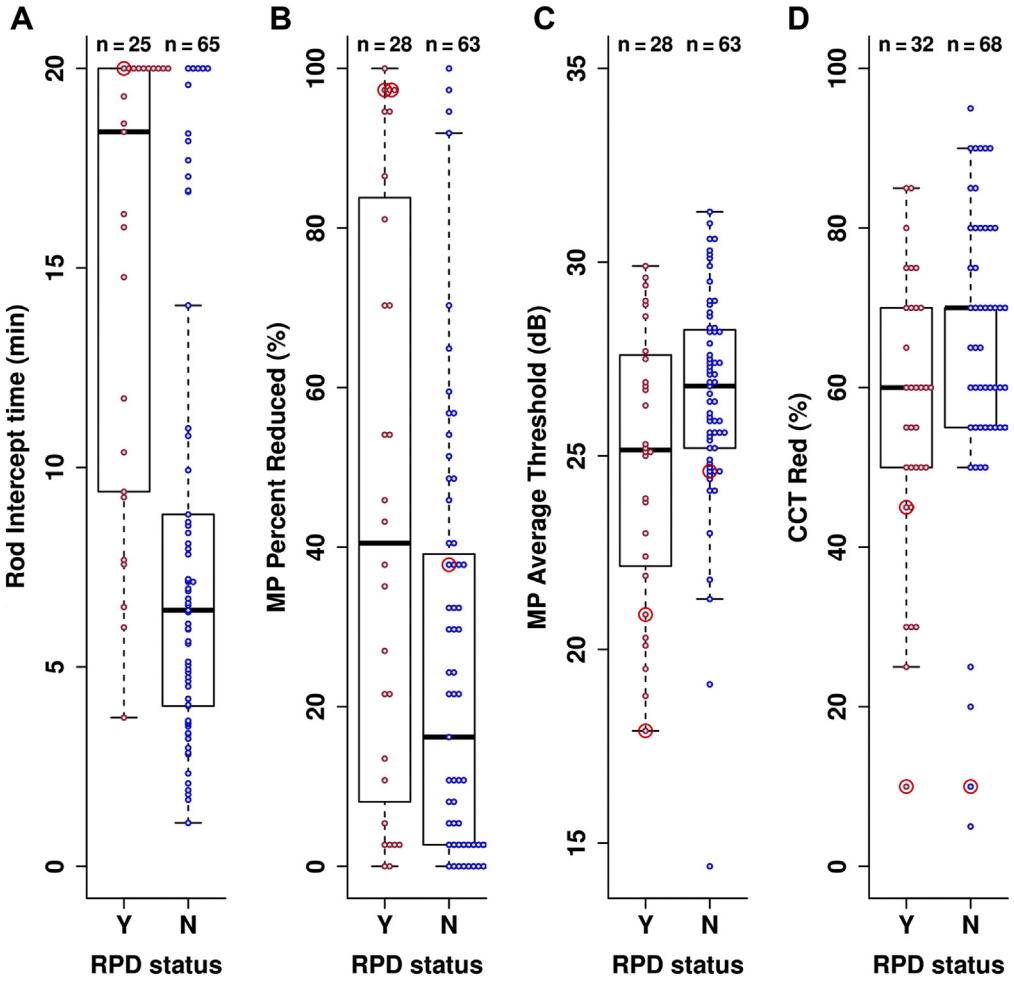

Figure 5. Performance of visual function (VF) variables stratified by presence (Y) or absence (N) of reticular pseudodrusen (RPD) on spectral domain OCT (SD-OCT) images in the study eyes at baseline. **A,** Dark adaptation rod intercept. **B,** Microperimetry (MP) percent reduced threshold (PRT). **C,** MP average threshold (AT). **D,** Cone contrast test (CCT) red. **Red circles** indicate the data from the 3 intermediate age-related macular degeneration (AMD) subjects who converted to neovascular AMD. dB = decibels.

risk alleles and 2 important SD-OCT biomarkers characterized at baseline, RPD and HRFs, may be predictors of AMD diagnosis at baseline or longitudinal VF performance.

Overall, we showed that although AMD progression is slow and appears not to be linear over the study period of 24 months, the 3 functional variables MP PRT, CCT red, and DA RIT that most consistently and significantly distinguished disease stages in cross-sectional analyses were also the VF metrics that most convincingly detected significant longitudinal VF changes in iAMD over a period of 24 months. In addition, we demonstrated that the 2 SD-OCT structural features HRF and RPD are moderately associated with VF decline measured by DA. Our results indicate that the presence of HRF or RPD on SD-OCT in the retina at baseline may not only impact disease staging based on structural features but also correlate with early VF deficits, suggesting a structure−function relationship that may already develop during earlier stages of AMD.

In patients with eAMD and iAMD, low luminance questionnaire scores were associated with LLVA and LLD measures on a computerized test.[33] Wu et al[8] showed that in patients with bilateral iAMD, LLD was correlated with self-reported symptoms on a 10-item Night Vision Questionnaire. However, LLD was not associated with the time to progression from iAMD to late disease over 36 months.[8] In 292 individuals with bilateral large drusen in the Laser Intervention in the Early Stages of AMD study, LLVA, MP, and BCVA demonstrated limited performance for detecting the earliest onset of neovascular and atrophic AMD. In our study, the lack of significant longitudinal change in LLVA and LLD at 12 months[21] and 24 months further highlights the need for very sensitive psychophysical measures with the ability to detect and follow functional impairments in the eAMD disease stages.

Wu et al[8] previously demonstrated that MP pointwise sensitivity SD, but not LLD, was significantly and independently associated with time to develop late AMD in 140 participants with iAMD over 3 years. However, this study observed that both functional measures were suboptimal at predicting progression,[13] similar to structural

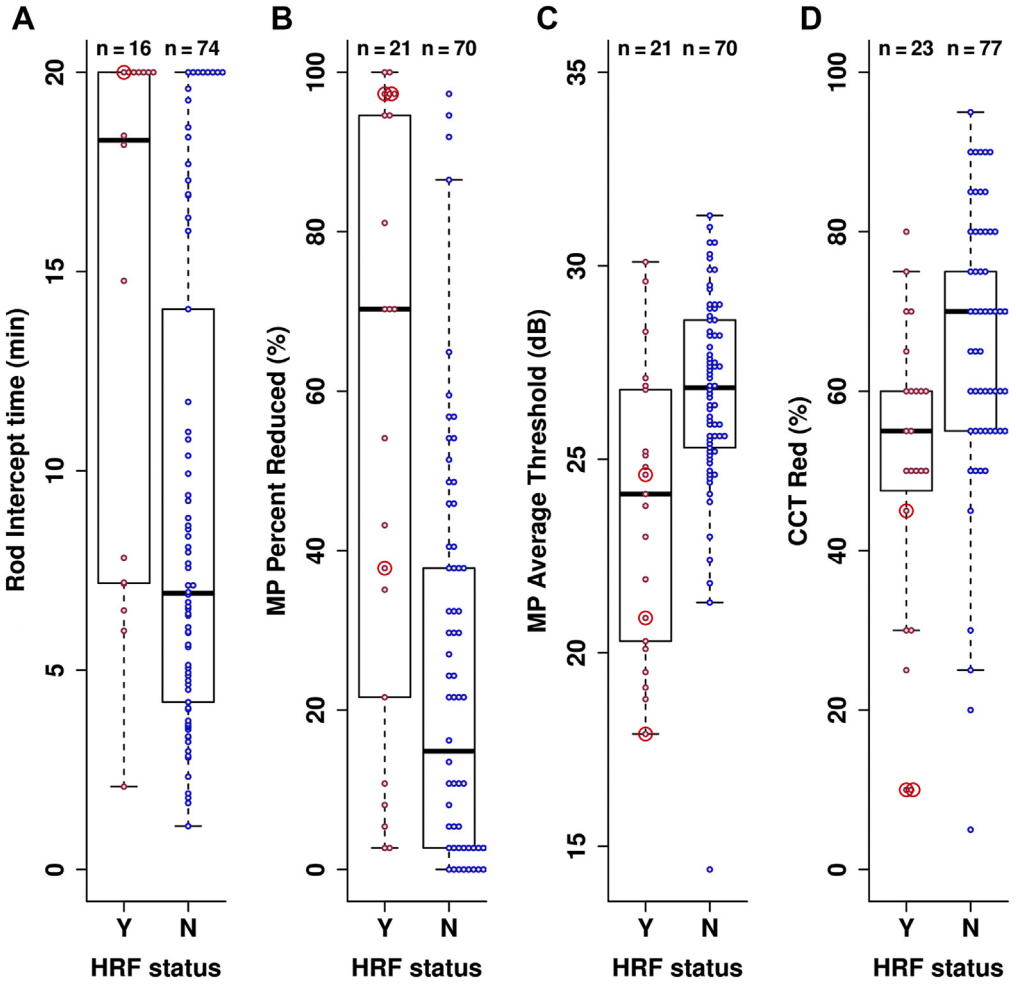

Figure 6. Performance of visual function (VF) variables stratified by presence (Y) or absence (N) of hyperreflective foci (HRF) on spectral domain OCT (SD-OCT) images in the study eye at baseline. **A,** Dark adaptation (DA) rod intercept. **B,** Microperimetry (MP) percent reduced threshold (PRT). **C,** MP average threshold (AT). **D,** Cone contrast test (CCT) red. **Red circles** indicate the data from the 3 intermediate age-related macular degeneration (AMD) subjects who converted to neovascular AMD. dB = decibels.

changes on color fundus photographs.[17] In our prior work,[20,21] we have studied longitudinal progression in 2 additional MP metrics, mean sensitivity across the grid (AT) and PRT, defined as the percentage of abnormal retinal sensitivity threshold below 25 dB. These indices are reported by the MAIA devices for clinical use. In line with the interpretation of these 24-month longitudinal analyses, as well as with the result of our 12-month publication by Hsu et al,[21] mesopic MP variables distinguished iAMD from normal controls at 12 months and all subsequent time points, as well as showed a significant deterioration relative to baseline. This is in line with recent research that provided evidence that mesopic MP PRT, defined as the percentage of abnormal retinal sensitivity threshold below 25 dB, significantly correlated with drusen volume for eAMD and iAMD subjects after controlling for age, presence of sub-retinal drusenoid deposits, and AMD stage.[34] Furthermore, our results also support the suggestion by Wu et al that mesopic MP may detect VF changes associated with iAMD progression and may correlate to microstructural changes in iAMD subjects.[18,35] In combination, the findings of the these studies support the robustness and validity of this psychophysical test as a sensitive measure of iAMD and the existence of a meaningful structure−function relationship in eAMD stages.

Prior studies have supported the concept that color discrimination distinguished eyes with iAMD from eyes with less severe AMD or normal aging changes.[36,37] Cone-adaptational kinetics were affected in eAMD and iAMD more than steady-state thresholds.[3] Intermediate AMD was associated with reduced sensitivity in foveal cone color and luminance channels, which was greatest for S-cones responsible for perception of blue color.[4] Cheng and Vingrys[5] also identified yellow-blue color deficits in eAMD subjects. In addition, the change in cone DA and yellow-blue chromatic sensitivity generally distinguished between AMD severity groups in 100 study participants.[7] It is important to note that McKeague et al[6] provided evidence of a learning effect with the Color Assessment and

Table 13. Association of Selected Visual Function Test Variables at 12 and 24 Months with 9 Selected Individual Genetic AMD-related Variants (SNPs)

Visual Function	Visit	SNP	Gene	*P* Value	Regression Model
CCT Red	12 mos	rs570618.T	CFH	0.0196	Extended model
LLD	12 mos	rs12930861.G	ATF7IP2	0.0114	Basic model
LLD	24 mos	rs1894596.C	TNR	0.0008	Extended model
LLVA	24 mos	rs1894596.C	TNR	0.0001	Extended model

CCT = cone contrast test; LLD = low-luminance deficit; LLVA = low-luminance visual acuity; SNP = single nucleotide polymorphism.
The analysis was based on a linear model controlling for covariates including baseline score, age, coronary artery disease, dry eye, and phakic status. Significance level reported is based on an uncorrected *P* value of $P < 0.02$ without multiple testing correction.

Diagnosis test, suggesting that clinical trials using color vision testing should ensure that sufficient training of participants is used.[6] Our study used a newer methodology based on cone contrast measurements performed after adequate pretest training. In our analysis, CCT red and CCT green showed some significant longitudinal deterioration relative to baseline, whereas CCT blue did not, because perception of the blue color is affected by cataract status.[22] These findings suggested that in particular the CCT red assessment may be most sensitive to detect such cone-related functional deficits in the eAMD stages. However, there is limited evidence on CCT evaluation in AMD subjects; thus, it is difficult to understand the underlying reasons for the differences among CCT red, CCT green, and CCT blue assessments. However, in our previous pilot study with smaller subject numbers, similar results have been obtained in the iAMD group, in which phakic status was noted as a potential confounder.[22] Therefore, phakic status was now included as an important covariate in the analysis of the longitudinal study, confirming these early results and increasing the validity of the current CCT findings.

In our study, DA RIT significantly differed between iAMD and normal control subjects, as well as between iAMD and eAMD subjects and thereby confirmed a problem with DA in the early stages of nonexudative AMD. This observation is consistent with a previous cross-sectional study in nonexudative AMD by Jackson et al[28] that found a direct association between DA RIT with AMD severity, such that every minute added to RIT during DA testing increased the odds of a subject having iAMD by 11.9%. Our results are also in agreement with prior studies by Dimitrov et al,[38,39] who demonstrated that 2 adaptation measurements (cone photo-stress recovery rate and rod DA recovery rate) were significantly abnormal in patients with intermediate drusen and that rod DA recovery had good diagnostic capacity in eAMD.

Remarkably, our categorical analysis of the DA RIT data also provided evidence for a longitudinal deterioration of this VF metric, which was suggested by an increasing proportion of "poor performers" in the iAMD group over time after the 6-month study time point. These data supported the concept that DA testing was able to provide a classification of iAMD poor performers by identifying not only a functional DA deficit at baseline but also a longitudinal change within the iAMD disease group based on a categorical analysis within the timeframe of 18 to 24 months. These observations thus supported the previously reported functional impairment of rod photoreceptors, as well as the progressive deterioration of DA RIT VF from eAMD to iAMD. These results are also in line with the observation by Owsley et al[29] that baseline delayed RIT in normal subjects correlated with a doubling of the risk of eAMD incidence 3 years later. Further validation of this work will be required. However, the preferential loss of the "poor performers" in the iAMD group between baseline and 6 months highlighted the need for careful attention being paid to the examinations from subjects who show prominent functional deficits on DA testing and for development of robust standardized operating procedures to allow for DA to be considered as an outcome measure in future clinical trials.

The existence of a potential structure—function relationship in the early stage of AMD is further supported by our observation of a significant moderate correlation between the presence of RPD and HRFs at baseline with DA RIT deficits at 12 and 24 months. These observations are in line with the recent cross-sectional study in eAMD and iAMD

Table 14. Status of RPD and HRF on Retinal Images in the Study Eye at Baseline by Disease Stage

AMD Diagnosis	RPD Positive/Total No. of Study Eyes (% RPD Positive Eyes)	HRF Positive/Total No. of Study Eyes (% HRF Positive Eyes)
Normal	0/21 (0%)	0/21 (0%)
eAMD	4/33 (12%)	3/33 (9%)
iAMD	28/47 (60%)	20/47 (43%)

AMD = age-related macular degeneration; eAMD = early age-related macular degeneration; HRF = hyperreflective foci; iAMD = intermediate age-related macular degeneration; RPD = reticular pseudodrusen.

subjects by Echols et al[40] that suggested a relationship between the presence of HRF and smaller hyperreflective specifications and rod-mediated DA deficits in the earlier stages of AMD.

It is interesting to note that in our study and in the work by Echols et al,[40] rod-mediated DA appears to show a closer relationship with the presence of the 2 structural biomarkers than with cone-rod–mediated VF tests (MP PRT, AT, CCT red). This may be indicative of an early RPE deficit that, together with associated inflammation, may contribute to HRF presence and may reflect reduced replenishing of the rod visual pigment via the visual cycle in the early stages of AMD.[40]

Another important observation emerging from this study was that the genetic burden score, which is based on the 9 most prevalent genetic risk factors for AMD, provided some thought-provoking insights despite the relatively small number of participants in our study. We confirmed that subjects with an increasing number of risk alleles had more advanced disease, as reflected by the significant association of the genetic burden score with the AREDS-based disease staging. This result validates the use of the genetic burden score in our study and supports the validity of the moderate correlation of the genetic burden score with DA RIT. Likewise, as observed for the structural biomarkers HRF and RPD, the 2 mesopic MP variables only showed a modest or nonsignificant correlations with the genetic burden score. The genetic analysis also uncovered 4 suggestive associations between VF variables (LLVA, LLD, CCT red) and specific SNPs (CFH, ATF7IP2, and Tenascin R). These observations, if confirmed in larger studies, may indicate that the genetic risks structurally defined not only earlier disease stages but also VF in AMD. These data raise the possibility that some of the pathogenic processes involved in AMD, such as RPE deficiency or inflammation described earlier, contribute to the rod-mediated DA deficit in early AMD disease stages and may be modified by underlying genetic risk variants, in particular those affecting the complement system (CFH) or the extracellular matrix homeostasis (Tenascin R). However, currently, the underlying molecular link between these genes and associated VF tests (LLVA, LLD, CCT red) remains unclear and speculative.

Study Limitations

There were several limitations to this study. The first is the use of AREDS categories for classifying the participants, rather than the Beckman Initiative Classification system. At the time of the study design, the decision was to use AREDS categories as an easily applicable scoring system based on the AREDS Reports[2,41] that would be familiar to most clinicians, including comprehensive ophthalmologists and optometrists who care for patients with early stages of AMD and normal, non-AMD individuals. As the AREDS Report No. 18 report suggested,[41] eAMD characteristics on examination and fundus photographs can be readily identified on ophthalmoscopy, slit-lamp biomicroscopy, and standard color photographs. One of the important goals of our study was to establish a clinically meaningful, easily adoptable protocol that would increase the likelihood for a low screen failure rate for classifying individuals with eAMD and iAMD. This would facilitate efficient recruitment in future large-scale trials and result in high agreement between sites and reading center confirmation during patient screening.

The second limitation is the sample size of 101 subjects enrolled was further reduced by the 30% dropout rate at the 24-month follow-up visit, mainly due to health problems not related to AMD. Additionally, 4 participants were removed from the study after the 12-month follow visit due to conversion to a different disease category (Table 1). We also noted that a subgroup within the iAMD group of 7 subjects in particular contributed significantly to the observed faster progression of VF deterioration during the first 12 months in our study. Of the 70 individuals remaining in the study at 24 months, some participants were unable to complete all psychophysical assessments and thus reduced statistical power for each VF measures. A study period of longer than 24 months or a significant higher number of study participants will be needed to reveal additional significant differences among study groups and longitudinally that can further characterize the earlier stages of AMD and confirm our observations in this study. In addition, DA testing in this study was stopped after 20 minutes to decrease testing burden in our aging cohort undergoing a number of psychophysical tests. This limited the RIT data collected by a ceiling effect. To mitigate this limitation, we performed an additional categorical analysis aimed to identify subjects with poor performance in DA in all 3 groups at all study time points. Finally, because only 3 of 31 iAMD eyes converted to neovascular AMD over the period of 24 months, no firm conclusions could be drawn regarding the predictive power of any of our observations for the development of late stage AMD, which would require significantly higher numbers of observations of conversion events.

Despite these limitations, our study has significant strengths. The described Duke FEATURE study is a comprehensive observational natural history study of VF assessments in eAMD and iAMD patients, including DA, CCT, BCVA, MP, and LLVA, which preceded and informed the current MACUSTAR initiative in Europe.[23,24] In addition, it provides both cross-sectional and longitudinal analyses of psychophysical measures across 24 months, while adjusting for covariates such as baseline status, age, coronary artery disease, dry eye, and phakic status, as well as considering the gender, race, medical history, and smoking status.

Conclusions

Evaluating these observations in larger prospective longitudinal studies will help validate the use of the most promising functional variables identified in this study (DA RIT, MP PRT, MP AT, and CCT red) and structural features (HRF and RPD) as potential clinical end points or population stratification markers in proof-of-concept clinical trials of nonexudative AMD.

Footnotes and Disclosures

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All authors have completed and submitted the ICMJE disclosures form. The author(s) have made the following disclosure(s):

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This work was submitted and accepted in abstract form to the Association for Research in Vision and Ophthalmology Virtual meeting, May 3−7, 2020, Baltimore, Maryland, and was presented at the Dry AMD Therapeutic Development Virtual Summit, October 28−29, 2020. It was not previously presented or is under consideration for presentation at any other meeting.

HUMAN SUBJECTS: Human subjects were included in this study.

This clinical study was approved by the Duke University Health System institutional review board, and was conducted in accordance with Good Clinical Practice following the guidance documents and practices offered by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use or applicable international regulatory authority laws, regulations, and guidelines. All patients signed a written informed consent prior to testing. This study abides by the tenets of the Declaration of Helsinki.

No animal subjects were used in this study.

AUTHOR CONTRIBUTIONS

Conception and design: Lad, Rautanen, Gayan, Stinnett, Luhmann

Data collection: Lad, Fang, Tessier, Luhmann

Analysis and interpretation: Lad, Rautanen, Gayan, Stinnett, Luhmann

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Overall responsibility: Lad, Fang, Rautanen, Gayan, Stinnett, Luhmann

Abbreviations and Acronyms:
AMD = age-related macular degeneration; **AREDS** = Age-Related Eye Disease Study; **AT** = absolute threshold; **BCVA** = best-corrected visual acuity; **CCT** = cone contrast test; **CFP** = color fundus photography; **DA** = dark adaptation; **dB** = decibels; **eAMD** = early AMD; **HRF** = hyperreflective foci; **iAMD** = intermediate AMD; **LLD** = low-luminance deficit; **LLVA** = low-luminance visual acuity; **MMRM** = mixed-effect repeated measure; **MP** = microperimetry; **PRT** = percent reduced threshold; **RIT** = rod intercept time; **RPD** = reticular pseudodrusen; **RPE** = retinal pigment epithelium; **SD** = standard deviation; **SD-OCT** = spectral domain OCT; **SNP** = single nucleotide polymorphism; **VF** = visual function.

Keywords:
Early AMD, Intermediate AMD, LLVA, Microperimetry, Visual function.

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