Longitudinal Changes in Retinotopic Rod Function in Intermediate Age-Related Macular Degeneration

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PURPOSE. Although impairment of rod function in the early stages of age-related macular degeneration (AMD) has been well recognized, data on longitudinal changes in rod function at multiple retinal locations remain limited. This study investigated the longitudinal changes in retinotopic rod function in eyes with intermediate AMD (iAMD).

METHODS. Complete ophthalmic examination, multimodal imaging, and scotopic perimetry were performed at baseline and at 12-month follow-up. Perimetric scotopic retinal sensitivities for the 505-nm stimulus were repeatedly measured for 20 minutes after exposing to a single photobleach (~30%). The rod intercept time (RIT) and retinal sensitivity at seven retinal loci within the central 12° were ascertained. Using the 95% limit of measurement variability derived from the control eyes as a reference, the proportion of test points with a significant change in retinal sensitivity or RIT at follow-up was determined.

RESULTS. Twenty iAMD and 6 control eyes were included. Decline in rod function was detected at 12-month follow-up in eyes with iAMD, but not in control eyes. Approximately 25% of test points in iAMD eyes showed a significant increase in RIT compared to 6% of test points with a decrease in RIT over the 12-month period (P < 0.001). Similarly, 40% of test points demonstrated a reduction in retinal sensitivity compared to the 7% of test points with an increase in retinal sensitivity at follow-up (P < 0.001).

CONCLUSIONS. There are detectable retinotopic changes in rod function over 12 months in iAMD eyes, indicating an ongoing disease progression in rod impairment or loss with time.

Keywords: age related macular degeneration, dark adaptation, rod function

Difficulties with night vision and a delay in adjusting from bright to dim lighting composed a common symptom in patients with the early stages of age-related macular degeneration (AMD), even when visual acuity (VA) is typically normal. The cause of this symptom appears to be associated with the impairment or loss of rod photoreceptors, as studies assessing rod function using dark adaptation show that rod-mediated dark adaptation (DA) parameters are markedly abnormal in eyes with early stages of AMD. In addition, histologic studies demonstrated a decline in rod density in AMD eyes. Studies have also shown that the magnitude of rod dysfunction increases with increasing AMD severity. Hence, rod function may have a potential role in monitoring AMD progression.

A study by Jackson et al. showed that patients with various stages of AMD exhibited a significant decline in rod function over a 12-month period, as measured by the rod intercept time (RIT), without changes in VA or clinically evident retinal structural changes. However, that study examined rod function at only a single retinal location, which may not be representative of the retina in general. Thus, the longitudinal changes in rod function at various retinal loci remain unknown in eyes with intermediate AMD (iAMD). Moreover, recent studies have shown that iAMD eyes with reticular pseudodrusen (RPD) exhibit greater DA impairment and are at increased risk of progression to late AMD than iAMD eyes without RPD. Hence, there is also a particular interest in exploring longitudinal and retinotopic changes in iAMD eyes with RPD.

We have recently reported in a cross-sectional study the retinotopic variation in rod function in subjects with iAMD, with the use of a dark-adapted chromatic perimeter (DACP; Medmont, Melbourne, Australia). In that study, we found that rod function was abnormal in eyes with iAMD but more so in iAMD eyes with RPD, and particularly within the central 6° of the retina. Thus, the purpose of this study was to examine the changes in rod function at multiple retinal locations in iAMD eyes with and without RPD over a 12-month period.

METHODS

The Human Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital approved this study. The research adhered to the tenets of the Declaration of Helsinki, and written informed consents were obtained following explanation of the clinical tests employed.

Subjects who had participated in our previous baseline cross-sectional study were invited to participate in this longitudinal study. All AMD subjects participating in the baseline study had an iAMD (all with drusen >125 μm, based upon the Beckman classification) with best-corrected visual acuity (BCVA) of 20/40 (logMAR 0.30) or better in both eyes. To
be eligible for this study, AMD participants were required to have remained clinically stable (i.e., retained their baseline’s Beckman grade of iAMD) over the review period. AMD subjects who progressed to late AMD in either eye were excluded from the study. Control subjects also had to remain free of any signs of AMD or other retinal diseases at 12 months follow-up. Participants attended a 12-month follow-up review for all clinical, imaging, and functional assessments.

**Examination and Imaging**

All clinical examination and imaging were performed after psychophysical tests to ensure no confounding bleaching of the retinal photopigment prior to DA tests. Subjects received a comprehensive ophthalmic examination, including BCVA with a modified version of the Early Treatment of Diabetic Retinopathy Study (EDTRS) chart and low luminance visual acuity (LLVA) following placement of a neutral density filter (2.0 ND) in front of the study eye.

Slit-lamp examination was performed on dilated pupils and cataracts were graded per the AREDS classification. Multimodal imaging (MMI) was performed and included color fundus photography (CFP) using a nonmydriatic fundus camera (Canon CR6-45NM; Canon, Saitama, Japan) and near-infrared reflectance (NJR), fundus autofluorescence (FAF), and spectral-domain optical coherence tomography (SD-OCT) imaging using a Spectralis HRA+OCT device (Heidelberg Engineering, Heidelberg, Germany). The AMD and RPD status was determined by a senior grader using the MMI images. RPD was graded into three categories of definitely present, questionable, or absent. The definitely present category was defined as the presence of ≥5 RPD on SD-OCT in more than one B-scan and at least one en face modality (CFP, FAF, NJR) or RPD present on two or more face modalities in the absence of SD-OCT findings (including outside the SD-OCT grid). RPD were considered questionable when questionably present in two modalities or if RPD was definitely present only on SD-OCT with no other en face image able to validate the OCT finding. RPD were considered absent if they were not present or did not reach the criteria for being present on any modality or were questionable on only one modality. In this study, only definite RPD cases were labeled as having RPD.

**Retinotopic Rod Function Assessment**

The protocol for assessing rod function at baseline study and 12-month follow-up was identical to allow comparison between the two results. Test protocol for obtaining dark-adapted perimetric retinal sensitivities for the 505-nm stimulus using a DACP has been reported previously. In brief, the study eye was dilated with 0.5% tropicamide and 1 minute after bleach.

The study eye was dilated with 0.5% tropicamide and 15 minutes after bleach. To determine the proportion of test points with a significant change in function, any test points at the follow-up visit that were greater than the 95% confidence interval of the change occurring in the control eyes were considered as a significant change in function. Changes in average PWS between the visits were determined using a linear mixed-effects model, with test visit as the fixed effect and test points nested within an eye as a random effect. Due to the majority of the test points in eyes with iAMD having a maximal RIT of 20 minutes, it was inappropriate to calculate the average for this parameter, and thus the average RIT were not used for the comparison.

**RESULTS**

Of the initial 30 participants who participated in the cross-sectional study reported in Fraser et al., 20 participants in the iAMD group (6 had RPD) and 6 in the control group performed both baseline and 12-month follow-up testing. One AMD patient was excluded from the study because of progression to advanced AMD; another AMD and two control baseline participants declined to participate in the longitudinal study for logistical reasons relating to transport access to the study center. The baseline data of these four subjects were not included in the analysis so that baseline and follow-up results were directly comparable. No AMD eyes without RPD at baseline developed RPD at follow-up. Those in the iAMD cohort had a mean follow-up time of 13.4 months (range, 11–16 months), compared to 13.0 months for controls (range, 11–16 months, P = 0.65).

The median age of the iAMD and control group at baseline was 72.3 (interquartile range [IQR]: 69.2–76.8) and 65.8 (IQR: 63.3–69.0) years, respectively (P = 0.013). Of the iAMD group, 6/20 eyes (30%) had RPD. There was no significant change in BCVA, LLVA, cataract lens grade, night vision questionnaire score, or clinical severity grade between baseline and follow-up in both groups (P ≥ 0.05, Table). However, the low luminance deficit (LLD) was improved in both groups at the follow-up visit (P ≤ 0.04, Table). This appeared to be associated with a slight improvement in the LLVA at follow-up.

Decline in rod functions was detected at 12 months follow-up in eyes with iAMD, but not in control eyes. For the RIT, there was no significant change in the mean RIT between baseline (6.3 ± 1.8 minutes) and follow-up visits (6.3 ± 1.4 minutes, P = 1.00) in the control group. As mentioned in the analysis section, the proportion of test points with a significant change in RIT was used for assessing the functional changes over time, rather than using the average RIT, because the majority of the test points in eyes with iAMD had a maximal RIT of 20 minutes. To determine the proportion of test points in the iAMD eyes with a significant change in RIT over time we performed a subanalysis comparing changes in rod function in iAMD eyes with and without RPD. Changes in rod function over time were determined by examining the changes in the average point-wise sensitivity (PWS) as well as the proportion of test points with a significant change in function. Any test points at the follow-up visit that were greater than the 95% confidence interval of the change occurring in the control eyes were considered as a significant change in function. Changes in average PWS between the visits were determined using a linear mixed-effects model, with test visit as the fixed effect and test points nested within an eye as a random effect. Due to the majority of the test points in eyes with iAMD having a maximal RIT of 20 minutes, it was inappropriate to calculate the average for this parameter, and thus the average RIT were not used for the comparison.

**Analysis**

Retinal sensitivities of the 505-nm stimulus for each retinal test point were plotted against time following bleach. The recovery was modelled using an exponential decay function. Two DA variables used for the analysis were the retinal sensitivity at 20 minutes after bleach and the RIT. The RIT was defined as the time required for sensitivity to recover to ~3.0 log units stimulus intensity. Retinal points that failed to recover to the criterion level at 20 minutes were assigned a maximal RIT of 20 minutes. The changes in retinal sensitivity and RIT between baseline and 12-month follow-up were determined and compared between iAMD and control groups, with a subanalysis comparing changes in rod function in iAMD eyes with and without RPD. Changes in rod function over time were determined by examining the changes in the average point-wise sensitivity (PWS) as well as the proportion of test points with a significant change in function. Any test points at the follow-up visit that were greater than the 95% confidence interval of the change occurring in the control eyes were considered as a significant change in function. Changes in average PWS between the visits were determined using a linear mixed-effects model, with test visit as the fixed effect and test points nested within an eye as a random effect. Due to the majority of the test points in eyes with iAMD having a maximal RIT of 20 minutes, it was inappropriate to calculate the average for this parameter, and thus the average RIT were not used for the comparison.
first calculated the test-retest variability limit of the RIT in the control eyes. The 95% confidence interval of the difference in RIT between the visits of the control eyes was ±3.4 minutes (Fig. 1A). Using this confidence limit as a reference, 25% (35/140) of test points in iAMD eyes showed an increase in RIT outside this normative range compared to 6% (8/140) of test points with a decrease in RIT (P < 0.001) over the 12-month period (Fig. 1C). There was also an increase in the proportion of test points in iAMD eyes failing to reach RIT at 20 minutes (baseline = 36% [51/140] compared to follow-up = 46% [64/140]); however, the increase was not statistically significant (P = 0.114, Fig. 2). Subanalysis comparing iAMD eyes with and without RPD showed that eyes without RPD had a greater proportion of test points with an increased RIT at follow-up (30%) compared to eyes with RPD (14%, P = 0.04, Fig. 1C). This was likely because the majority of test points in iAMD eyes with RPD had already reached the maximum RIT. The retinotopic variation in rod-mediated DA observed in the baseline study was once again demonstrated at follow-up, with the greatest change within the central 6° of the retina (Fig. 2). Representative RIT changes between baseline and 1-year follow-up in control and AMD cases are shown in Figure 3.

For the dark-adapted retinal sensitivity measured at 20 minutes after bleach, there was a trend of a decline in the average PWS at 12-month follow-up for the iAMD eyes (baseline PWS = 48.4 decibels [dB]; follow-up PWS = 45.8 dB, P = 0.054) but not for the control eyes (baseline PWS = 58.1 dB; follow-up PWS = 58.1 dB, P = 1.00). The 95% confidence limit of the difference in PWS between the visits for the control group was approximately ±5.0 dB (Fig. 1B). Using this normative limit as a reference, 40% (56/140) of iAMD test points demonstrated a significant reduction in retinal sensitivity outside the normative range, compared to the 7% (10/140) of iAMD test points with an increase in retinal sensitivity (P < 0.001) at follow-up (Fig. 1D). Subanalysis of iAMD eyes with and without RPD showed that the average PWS of eyes with RPD (38.5 dB) was significantly lower than that of eyes without RPD (48.0 dB, P < 0.001) at follow-up. However, the change in average PWS between baseline and follow-up was not significant for either eyes with RPD (baseline PWS = 41.7 dB; follow-up PWS = 38.9 dB, P = 0.258) or eyes without RPD (baseline PWS = 50.7 dB; follow-up PWS = 48.2 dB, P = 0.081).

**DISCUSSION**

This study examined longitudinal changes in rod function in iAMD, at multiple retinal locations, using a DACP over a 12-month period. Our study demonstrated a significant decline in rod photoreceptor function at multiple retinal loci in participants with iAMD at 12 months follow-up, but not in control participants. Rod function was further compromised in the iAMD group with RPD compared to the iAMD group without RPD. This occurred without observed changes in the clinical characteristics, BCVA, or LLVA in the study groups between baseline and follow-up. Consistent with our result, Jackson et al. demonstrated significant worsening of DA impairment in patients with AMD at 12 months follow-up, prior to clinically evident changes in acuity or retinal morphology. Our data, however, also allow us to conclude that this change occurs at multiple locations, with worse loci within the central 6° retina.

We and others have identified that iAMD eyes with RPD have significant delays in DA and reduced sensitivity. While we found a significant delay in RIT at follow-up, the exact magnitude of the increased RIT in eyes with RPD could not be estimated in our study because many of the test points were already severely abnormal and failed to reach the rod criterion level within the 20 minutes after an approximate 50% bleach. Nevertheless, iAMD eyes with RPD consistently had a greater proportion of test points with longer RIT or test points that failed to reach the criterion level compared to iAMD without RPD. As found in our baseline study, rod functions were particularly abnormal within the central 6° of the retina at follow-up, highlighting the need for testing at multiple retinal locations.

Despite significant advances in the treatment of neovascular AMD, there are still no specific treatments for nonneovascular AMD. One significant impediment to the investigation of novel interventions is the lack of sensitive biomarkers that could be used to follow the progression of AMD from its early stages to more advanced disease. A robust marker of disease severity that changes in a clinically relevant time course would greatly facilitate the implementation of trials to evaluate potentially novel treatments. As more sites internationally become familiar with perimetric methods, it becomes feasible to consider perimetry and adaptation kinetics as possible clinical tests in novel intervention studies. The results presented here suggest that it is possible to measure changes in rod function over a clinically reasonable time frame of 12 months and that rod function parameters could potentially be used as functional biomarkers for assessing the effectiveness of interventions in early stages of AMD.

The key strength of this study was the ability to determine rod function at multiple retinal locations within the same testing session. The instrument (DACP) used had a large dynamic range of stimulus intensity that covered the entire range of rod sensitivity, allowing subtle changes in sensitivity to be detected. To the best of our knowledge, this is the first longitudinal study to have examined the natural progression in rod function in patients with iAMD at multiple retinal locations. A weakness in our study was that our test protocol was unable to return the RIT for many test points in iAMD eyes, due to a floor effect, and thus we were unable to further analyze these data, such as to examine the proportion of RIT change as a function of eccentricity. Our test protocol could be further optimized to allow a measurable RIT to be obtained at all test points rather than timing out at 20 minutes. This

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**TABLE. Clinical Characteristics of the Participants at Baseline and 1-Year Follow-Up**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>Follow-Up</th>
<th>P Value</th>
<th>Baseline</th>
<th>Follow-Up</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA, median (IQR, letters)</td>
<td>88.0 (84.3–90.8)</td>
<td>88.0 (84.0–95.0)</td>
<td>0.98</td>
<td>89.5 (88.8–93.5)</td>
<td>86.5 (84.0–91.5)</td>
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<tr>
<td>LLA, median (IQR, letters)</td>
<td>73.0 (67.3–77.0)</td>
<td>77.5 (71.8–79.8)</td>
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<td>74.5 (70.5–76.0)</td>
<td>79.5 (77.3–80.0)</td>
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<tr>
<td>LLD, median (IQR, letters)</td>
<td>15.0 (12.0–18.8)</td>
<td>12.0 (10.0–14.0)</td>
<td>0.04</td>
<td>15.5 (14.5–19.5)</td>
<td>10.0 (4.8–12.5)</td>
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<tr>
<td>NVQ, median (IQR, raw score)</td>
<td>17.0 (12.5–22.5)</td>
<td>12.5 (10.3–22.8)</td>
<td>0.40</td>
<td>14.5 (10.8–18.3)</td>
<td>15.0 (11.0–20.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cataracts*, number (% of eyes)</td>
<td>8 (40)</td>
<td>10 (50)</td>
<td>0.65</td>
<td>3 (50)</td>
<td>4 (67)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* NVQ, night vision questionnaire.
* All with a score of 1. There was no change in cataract grading between the visits.
could be achieved by several methods, including varying the level of bleach, extending DA time, or introducing a red background. However, the feasibility of each of these methods required further investigations. Another limitation was that the study had a relatively small sample size. To minimize the effect of the small sample, we pooled the data of all seven test points from all six controls. Thus the total number of data points is 42 samples, and these control samples were used to calculate the normative range. Clearly, there is a need to undertake this type of study in larger AMD cohorts, recognizing that it is difficult to recruit participants for the somewhat long and arduous testing procedure. However, we found that despite a small sample size, a statistical significant change in rod function can still be detected while other clinical parameters remain unchanged. This suggests that our test protocol is sensitive enough to detect a change in rod function despite these small numbers. Finally, the study groups were not age-matched, with control subjects being younger on average. Nevertheless, the main focus of the study was to determine whether or not we could detect a change in rod function over a 12-month period in people with clinically stable iAMD. Thus the difference in age between the groups is not critical, as we were interested to compare someone’s performance on two occasions. The control participants allowed us to show that without disease, people do not change their function over a 12-month period.

In conclusion, we demonstrated retinotopic changes in rod function in eyes with iAMD over a 12-month follow-up period, and presented data on the normative range of this test protocol.

**Figure 1.** Scatter plots showing rod intercept time (A) and retinal sensitivity (B) at baseline and 12-month follow-up for the study groups. The dashed lines represent the 95% confidence interval of the difference in rod intercept time (RIT) between the baseline and follow-up visit in the control eyes. Any test points in eyes with intermediate AMD (iAMD) that fell outside the limits were considered as having significantly changed in function (improved or declined) from the baseline visit. There was a greater proportion of test points with declining function (declined) compared to the proportion of test points with improving function (improved) at 12-month follow-up, as indicated by the RIT (C) and retinal sensitivity (D).
during which no change in the clinical severity occurred nor did changes in BCVA or LLVA. Our results highlight an ongoing disease progression in rod impairment or loss with time in eyes with iAMD, particularly those with RPD. Regional variation in rod function found at baseline was also observed at follow-up, which highlights the need for testing rod function at multiple locations.

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**FIGURE 2.** Rod intercept time (RIT) at various retinal loci, at baseline and follow-up. Many of the test points in iAMD eyes had an increase in RIT, with the majority of test points with worsening RIT at follow-up located within the central 6° of the retina. The proportion of test points that did not reach the rod criterion level after 20 minutes of dark adaptation was approximately 36% at baseline, which was increased to 46% at the follow-up.

**FIGURE 3.** Representative changes in rod intercept time (RIT) between baseline and 12-month follow-up visit in a control eye and in AMD eyes with and without reticular pseudodrusen (RPD). There was a minimal change in RIT in the control eye; however, the RIT of many test points was markedly increased at follow-up in AMD eyes. Note that in eyes with RPD, four test points within the central 6° of the retina already reached the maximum RIT of 20 minutes, and thus it was not possible to determine whether there was any worsening of rod function at these retinal loci.
References


