tvst

Retina

Assessment of Scotopic Function in Rod–Cone Inherited Retinal Degeneration With the Scotopic Macular Integrity Assessment

Jasleen K. Jolly[1–4], Anika Nanda[1,2], Thomas M. W. Buckley[2], Maximilian Pfau[5,6], Holly Bridge[3], and Robert E. MacLaren[1,2]

[1] Nuffield Laboratory of Ophthalmology, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK
[2] Oxford Eye Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, UK
[3] Oxford Centre for Functional MRI of the Brain (FMRIB), Wellcome Centre for Integrative Neuroimaging, University of Oxford, Oxford, UK
[4] Vision and Eye Research Institute, Anglia Ruskin University, Cambridge, UK
[5] Department of Ophthalmology, University of Bonn, Bonn, Germany
[6] Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD, USA

Correspondence: Jasleen K. Jolly, Vision and Eye Research Institute, School of Medicine, Anglia Ruskin University, Young Street, Cambridge CB1 2LZ, UK.
e-mail: jasleen.jolly@aru.ac.uk

Received: December 7, 2021
Accepted: December 29, 2022
Published: February 7, 2023

Keywords: rod–cone dystrophy; scotopic function; microperimetry; visual field; MAIA

Citation: Jolly JK, Nanda A, Buckley TMW, Pfau M, Bridge H, MacLaren RE. Assessment of scotopic function in rod–cone inherited retinal degeneration with the scotopic macular integrity assessment. Transl Vis Sci Technol. 2023;12(2):10, https://doi.org/10.1167/tvst.12.2.10

Purpose: The scotopic macular integrity assessment (S-MAIA) can perform scotopic assessment to detect localized changes to scotopic rod and cone function. This study is an exploratory investigation of the feasibility of using the S-MAIA in a rod–cone dystrophy population to identify the pattern of loss in scotopic photoreceptor function.

Methods: Twenty patients diagnosed with a rod–cone dystrophy underwent visual acuity testing, full-field stimulus threshold assessment, and multiple S-MAIA tests after dark adaptation periods of 20 minutes and 45 minutes performed separately. Only right eyes were tested. Three tests were performed following a learning test. A Bland–Altman analysis was used to assess repeatability and agreement between tests after the two time periods. Spatial interpolation maps were created from the group plots to display the pattern of rod and cone loss.

Results: Learning effects took place between testing sessions 1 and 2 but not 2 and 3. Limits of agreement were larger in the patient eyes than control eyes, but within previously reported values. Using longer adaptation time of 45 minutes did not offer a significant advantage over 20 minutes. Patterns for the cyan and red sensitivities were different, indicating different patterns of loss for rods and cones.

Conclusions: A dark adaptation time of 20 minutes before testing is sufficient for thresholding. The S-MAIA is suitable for use in patients with a logarithm of the minimum angle of resolution vision of at least 0.7 and provides a viable outcome measure for patients with rod–cone dystrophies and preserved central vision. The spatial information about scotopic function from the S-MAIA provides information about disease processes and progression.

Translational Relevance: There is a need for scotopic measures for use in clinical trials. Scotopic microperimetry works well in patients with early disease, allowing the extension of recruitment criteria for novel therapies of rod–cone dystrophies.

Introduction

Rod–cone dystrophies (RCD) comprise a diverse group of inherited retinal degenerations. Clinically, RCD is characterized by early loss of night and peripheral vision with subsequent loss of central vision. Recently, important advances toward the treatment of RCD have been made, including potential therapeutic approaches such as gene therapy.[1,2] Thus, measuring scotopic vision forms an important outcome measure of photoreceptor function

under scotopic conditions—the luminance that is most affected most in RCDs. Dark adaptometry and full-field stimulus threshold (FST) measurement are useful as global measures of dark adaptation across the retina.[3] Although Roman et al.[4] showed it does not correlate well with standard automated perimetry, Dimopoulos et al.[5] did suggest that the central retinal plays a larger contribution to the FST response. Hence, the relationship with microperimetry may be stronger than with traditional perimetric methods. Because both FST and microperimetry are key outcome measures in gene therapy trials,[6] a direct comparison of the two techniques can help to make an informed decision of the limitations of each and, therefore, when they are appropriate to use. In early disease or after interventional therapies, it can be useful to understand the localized changes in the central retina. Two-color perimetry has been used to investigate the relative contributions of rods and cones under multiple light conditions.[7–10] These results showed that both the stimuli and background needed to be manipulated appropriately to differentiate between rod and cone contributions to vision. Using spatial information allows the pattern of loss to be detected, which can provide information into disease processes[11–14] and better monitoring of early disease.

In principle, dark-adapted sensitivity to short-wavelength stimuli may be evaluated using commercially available perimetry devices.[15,16] However, in patients with both stable and unstable fixation, gaze-contingent testing (so-called microperimetry or fundus-controlled perimetry) is preferable to ensure accurate stimulus placement during and across examinations.[17,18] Detailed measurement of the visual fields can be performed with devices such as the macular integrity assessment (MAIA) (Centervue, Padova, Italy) but standard protocols are performed under mesopic conditions. Recently, the MAIA device has been updated and is now capable of performing scotopic testing of both rods and cone, (known as the scotopic MAIA [S-MAIA]).[18] For a clinical trial setting, using an existing device approved by the US Food and Drug Administration provides advantages regarding the acceptability of the results produced. The S-MAIA uses cyan stimuli (505 nm) and red stimuli (627 nm) with the difference calculated between them. The original version had a decreased decibel range of testing under scotopic testing, but this range has since been expanded to the full 36 dB in all conditions to remove any ceiling effects and allow lower intensity lights to be presented.[18] However, the decibel scale used for scotopic tests is not directly comparable with the mesopic tests because it uses the scotopic candela per square meter scale. Both the cyan and red stimuli are thought to be rod mediated in healthy observers outside of the central retina, which features the rod-free zone. The device is calibrated according to the scotopic luminosity function. Thus, the expected cyan–red sensitivity difference would be 0 dB for loci with normative rod function.[19] Because the dark-adapted cone sensitivity is close to the rod sensitivity for the red long wavelength stimulus, isolated rod dysfunction is characterized by a predominant loss of scotopic cyan sensitivity, resulting in negative cyan–red sensitivity differences.[4,20,21] The S-MAIA has been used in the investigation of Bruch's membrane diseases, such as age-related macular degeneration and pseudoxanthoma elasticum, and was shown to allow for the identification of loci with predominant rod dysfunction.[22–24] However, the device has not been evaluated in patients with RCDs to date.

The dark adaptation time required before testing has been arbitrarily set to 30 to 35 minutes in previous work. However, preliminary findings from a study in a healthy cohort indicated 20 minutes was sufficient.[25] This dark adaptation time is used for scotopic electroretinography and is considered sufficient for scotopic testing.[26] However, previous work has shown that dark adaptation times are significantly delayed in eyes with rod–cone degeneration, so 20 minutes may not be sufficient and may need to be extended to 45 minutes.[27] This study has two key aims: first, to characterize results, including repeatability, from S-MAIA in a heterogeneous group of patients with RCD compared with control participants and, second, to investigate whether the duration of pretesting dark adaptation (20 minutes vs. 45 minutes) affected the results. The comparison against FST results shows that the spatial results from microperimetry cannot be extracted from a global measure of scotopic function. Currently, FST is the only scotopic functional measure used in clinical trials, but this is not sufficient for all trial types. This factor makes this work important for demonstrating the usefulness of S-MAIA as a potential outcome measure.

Methods

Because this study was exploratory, a mixed group of patients with RCD was recruited. The only requirement for participation was the ability to see the targets. RCD was defined as primary rod disease and could be diagnosed based on genotype and/or phenotype. Age- and sex-matched control participants were recruited from a pool of volunteers. Ethical approval for the study was obtained from the Health Regulatory

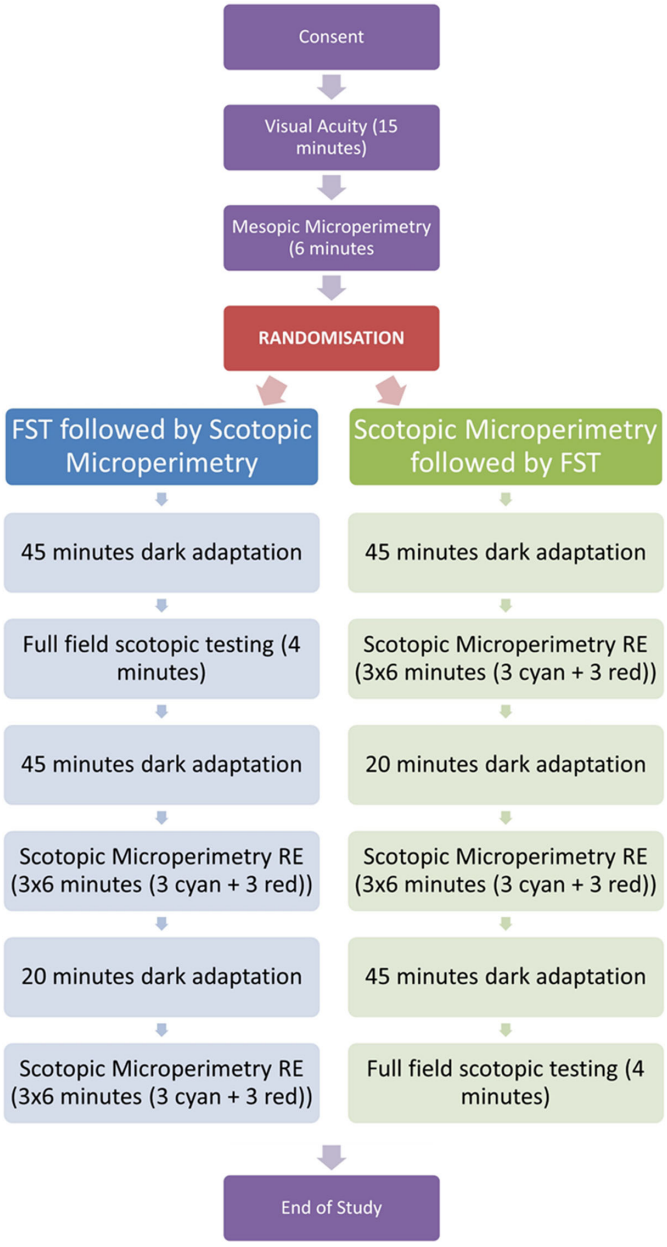

Figure 1. Flowchart of study assessments.

Authority (REC reference 18/WM/0086). The study adhered to the Declaration of Helsinki and informed consent was obtained.

Each participant underwent Early Treatment Diabetic Retinopathy Study visual acuity testing and a training test under mesopic conditions. The participants were randomized to FST or scotopic MAIA via a preset order to account for fatigue effects (Fig. 1). Mesopic microperimetry using the scotopic central testing grid was conducted after 5 minutes of adaptation.[28] Then, after a further 45 minutes dark adaptation, S-MAIA testing (Centervue) or white FST using the Espion E2 module (Diagnosys LLC, Lowell, MA, USA) was undertaken. S-MAIA sessions involved testing with cyan and red lights with wavelengths of 505 and 627 nm, respectively, and had a sensitivity range of 0 to 36 dB. The sessions consisted of three repetitions of a cyan test first, followed by three repetitions of a red test, with a recovery period of a minimum of 3 minutes in between each test repetition to allow calculation of repeatability. The participants were given a rest break under normal light exposure to improve reliability. Then, they were asked to return for 20 minutes dark adaptation followed by the final S-MAIA test (Fig. 1). All follow-up testing was conducted using the follow-up function to ensure consistency of points being tested. The grid chosen consisted of 37 points and covered the central 10° to include the region of the retina where rod density begins to increase.[29] The grid configuration is visible in Figure 5, which shows the output of the test result.

All assessments were conducted on the right eye only to avoid fatigue. Adjustment for bilateral testing was, therefore, not required. No dilation was used as long as the minimum exit pupil was achieved.[30] A Bland–Altman analysis was conducted comparing test 1 and test 2 followed by test 2 and test 3. For point-wise repeatability analysis, results in which at least one result of less than 0 dB at a given loci across the three tests were excluded to account for floor effects.[31] The groups were compared for each parameter using the Mann–Whitney *U* test with Bonferroni correction for multiple comparisons. All statistics were conducted in SPSS (version 25.0, IBM Software, Armonk, New York, USA). To explore the spatial patterns, results were analyzed across subjects in patient and control groups and interpolated plots created using MATLAB (version 2018a, The MathWorks, Natick, MA, USA).

Results

Twenty patients with clinically confirmed RCD and 13 age-matched control participants were recruited. One patient could not perform any of the testing owing to poor vision and was excluded from the cohort. Eight patients had a clinical diagnosis of retinitis pigmentosa based on the phenotypic fundal appearance. One patient had a genetically confirmed mutation in the TOPORS gene, four had mutations in the USHERS2A gene, six had choroideremia mutations, and one had a mutation in RP2. Subject demographics and summary results of the participants completing the study are shown in Table. For cyan–red differences, a greater degree of negativity would point toward greater rod dysfunction.

Table. Subject Demographics and Summary Results, Including Repeatability Analysis

Measure	Patient Eyes	Control Eyes
Age (years)	47.7 [27–77]	48.0 [26–81]
Female/male	7/12	7/6
Visual Acuity (letters)	78 [48–90]	84 [76–93]
Mesopic MAIA threshold (dB)	11.7 ± 9.3	26.6 ± 1.5
Scotopic cyan threshold (dB)	6.1 ± 7.5	21.1 ± 4.9
Scotopic red threshold (dB)	9.2 ± 6.7	21.8 ± 3.4
Difference between cyan and red (dB)	−3.2 ± 4.9	−1.2 ± 1.1
FST (dB)	−27.3 ± 14.0	−51.7 ± 5.1
S-MAIA repeatability (full field)		
BA limits of agreement sessions 1 and 2 cyan (dB)	4.89 to −4.86	2.13 to −0.85
BA limits of agreement sessions 2 and 3 cyan (dB)	2.48 to −2.00	1.39 to −1.39
BA limits of agreement sessions 1 and 2 red (dB)	5.71 to −3.67	1.89 to −1.25
BA limits of agreement sessions 2 and 3 red (dB)	3.32 to −3.56	1.94 to −1.56

BA, Bland-Altman.
Data are presented as mean ± standard deviation or mean [range].

Repeatability

Repeatability was assessed on data collected after 45 minutes dark adaptation. Repeatability analysis with Bland–Altman shows that the limits of agreement decreased between testing sessions 2 and 3 compared with the first two sessions (Table), indicating that the first session acts as a learning experience for both colors, particularly in the patient population. The limits of repeatability were wider in the patient population compared with the control population (Table). Pointwise sensitivity was examined between sessions 2 and 3. The coefficient of repeatability for the cyan and red test protocols were 7.15 dB and 11.69 dB, respectively. Bland–Altman plots are shown in Figure 2. The mean bias between sessions 2 and 3 for cyan was 0.24 dB and less than 0.005 dB for patients and controls, respectively. For red it was −0.12 dB and 0.19 dB for patients and controls, respectively.

Group Comparisons

The data in each group are presented in Figure 3. S-MAIA values from session 2 were used for statistical analyses to allow for the learning effect. Visual acuity was not significantly different between groups ($P = 0.07$), but microperimetry threshold for mesopic, cyan, and red stimuli, as well as FST were significantly different between groups ($P < 0.01$).

FST measurements were calibrated in photopic units of candela per square meter, whereas the scotopic MAIA is calibrated on the scotopic candela per square meter scale. There is no simple formula to correct these scales, making direct comparisons difficult.[32] Additionally, one is on a negative scale and the other is on a positive scale. A direct comparison of the average threshold from dark-adapted cyan testing to the FST is not meaningful given, that the former measure would represent the average threshold across the central 10°. In contrast, FST results are derived from loci representing the most sensitive points from a wider area of the retina.[33] A comparison of FST thresholds with the mean threshold of the three most sensitive test points revealed a strong linear relationship with a (negative) slope of unity (slope = −1.05 dB FST/dB S-MAIA, ; intercept = −17.31 dB, ; $R^2 = 0.381$) (Fig. 4).

Spatial Patterns

Typical examples of test results from a control (left) and patient (right) eye are shown in Figure 5. The mesopic maps show normal function across the field in the control eye compared with a small central island in the patient eye. The cyan map shows a central scotoma related to the rod-free region in the healthy eye (Fig. 5B). The patient eye shows several regions with no apparent rod function. On the red sensitivity maps (pink-colored points), the control eye shows a uniform ability across the visual field, whereas the patient eye has patchy loss of scotopic cone function, which does not match the cone function loss under mesopic conditions (Fig. 5C). The difference map in the healthy control shows a central cone-dominated region. as would be expected due to the rod-free foveal zone (Fig. 5D). The patient eye shows different areas of dominance for rods and cones (Fig. 5D).

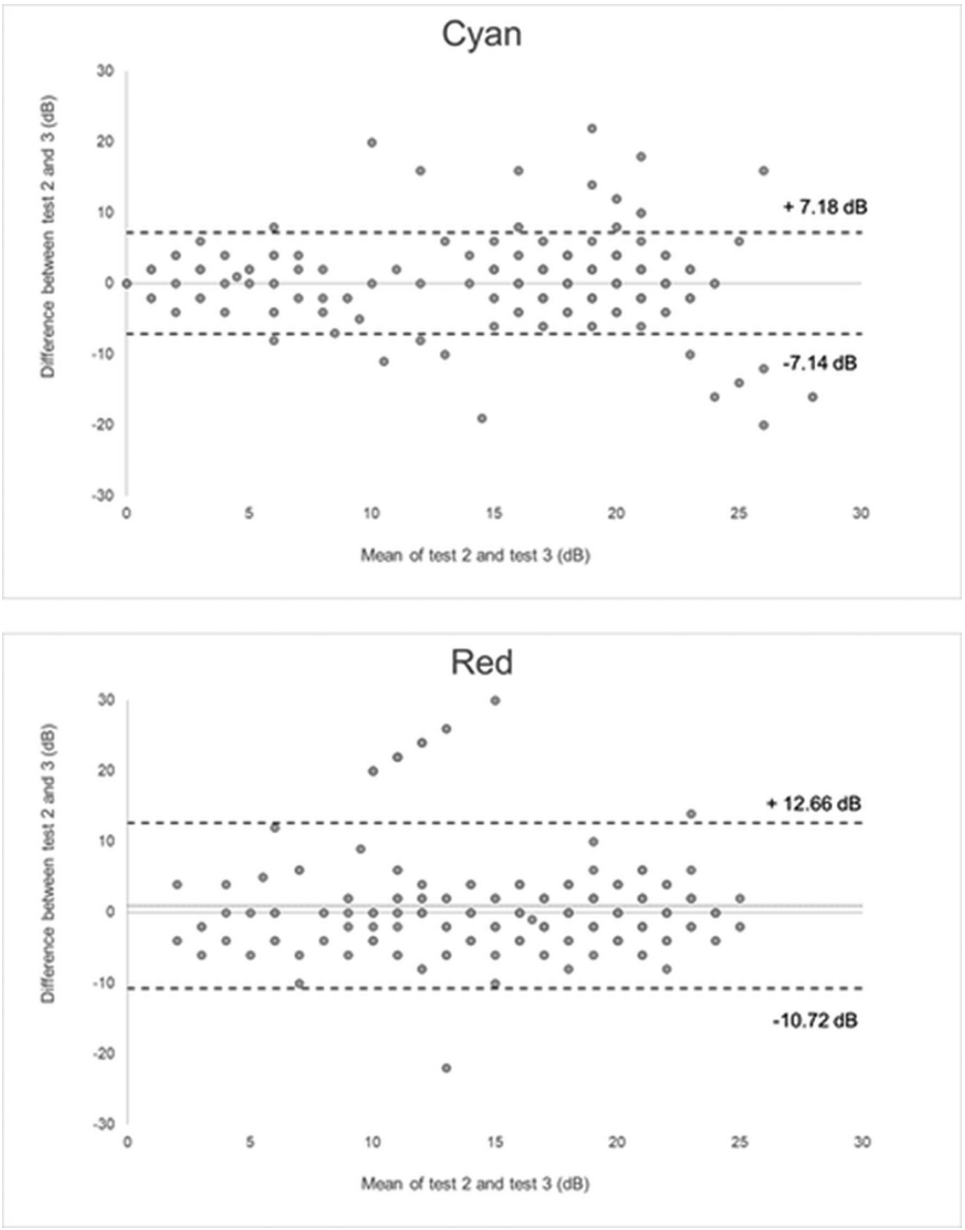

Figure 2. Bland–Altman analysis showing point wise analysis for the patient population. The dotted line represents the mean test–retest difference.

Importantly, the absence of a cyan–red difference (brown color) can represent either equal degrees (including total) loss of rod and cone function (in the context of severely decreased thresholds), or normative rod and cone function (in the context of normative cyan and red thresholds).

Interpolated group averages were plotted against the original color schemes for each test type (Fig. 6). Mesopic testing shows all points within the normal range with highest sensitivity at the central foveal point (Fig. 6A). The patient results show a marked generalized depression across the visual field with relative central preservation. Scotopic testing with cyan stimuli (rod function) reveals a central depression denoting the rod-free region in the control eyes. Sensitivity increased with increasing eccentricity. As expected, the patient population also showed the rod-free zone (Fig. 6D). Scotopic function was depressed across the field (Figs. 6B and C). However, the greatest depression was in the periphery with relative preservation around 2.5°. This point coincides with the region in which rod density begins to rise sharply.[34] The difference plots reveal a cone-mediated fovea in the control eyes surrounded by a balance between rod- and

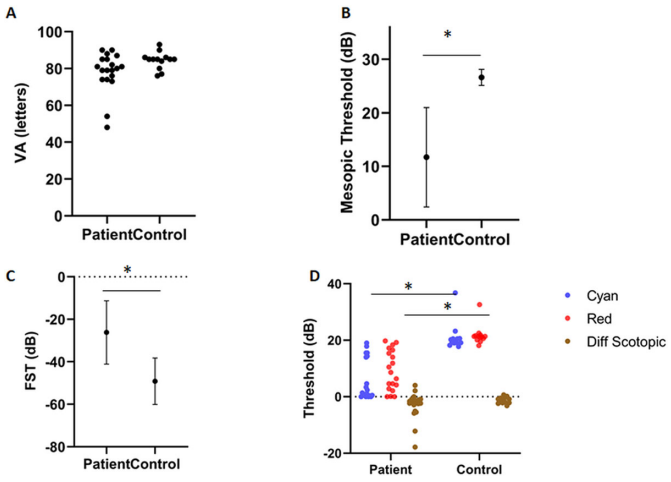

Figure 3. (A) Raw visual acuity measures for each in on control and patient groups. Boxplots of mesopic microperimetry threshold (B) and FST threshold (C) showing median, interquartile range and full maximum and minimum of datasets. (D) Mean of each S-MAIA test conducted with cyan and red stimuli with the difference between the tests shown for each group. * Statistically significant difference between groups ($P < 0.01$).

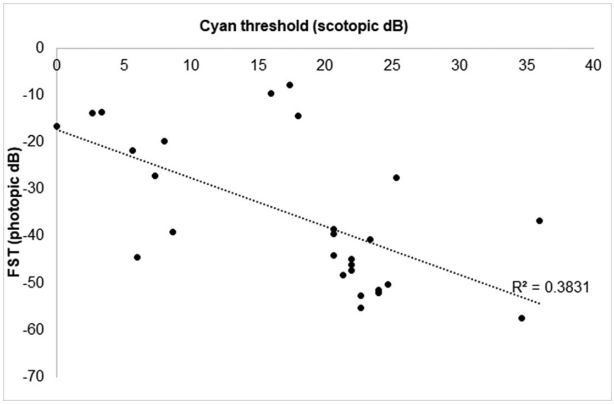

Figure 4. Association between the threshold at the three most sensitive loci (scotopic dB) in scotopic cyan microperimetry and FST measurements. There does appear to be a significant correlation (r = 0.62, ; $P < 0.01$). Of note, FST results are most likely driven by loci outside of the central 10° in healthy subjects and patients with mild disease, which were not evaluated using microperimetry testing in this study.

cone-mediated vision. The patient eyes have a larger zone of cone-mediated vision. Severe loss of both rod and cone function can result in a difference of 0, so the brown areas are less informative in this case.

A variety of phenotypes are shown in Figure 7 in eyes with a similar FST. The patterns of rod and cone loss vary across eyes with similar levels of vision loss and the same genotype, allowing for more detailed phenotyping of scotopic function that FST.

Dark Adaptation Time

The repeat testing after 20 minutes of dark adaptation was completed by 17 of the 20 patient participants. The mean difference between test session 2 at 45 minutes and the session performed at 20 minutes was 0.5 dB for cyan and −0.3 dB for red. When looking at the difference between the cyan and red tests at each time point, the values are 2.45 and 2.88 dB at 45 and 20 minutes, respectively. The Wilcoxon matched pair signed ranks test showed no significant difference between the time points ($P = 0.68$). The limits of agreement for cyan were −3.1 to 4.1 dB for cyan and −4.8 to 4.1 dB for red. Looking at the results from the individual eyes, the maximal differences between tests occurred with regard to the cyan stimuli, when thresholds were less than 7 dB (Fig. 8).

Discussion

The S-MAIA was able to differentiate between RCD and control eyes effectively. The repeatability assessment of the S-MAIA shows a larger coefficient of repeatability between session 1 and session 2 compared with between session 2 and session 3. This learning effect with the first test is in keeping with the pattern previously reported for mesopic microperimetry and indicates that the first session's results should be discarded.[35] The repeatability measures reported here between session 1 and session 2 are lower than those reported previously.[19,36] However, many of the participants in this study were not perimetry naïve, thereby effectively decreasing the learning effect. Additionally, all participants underwent a training field under mesopic conditions before starting the study assessments.

Participants were excluded if vision was too poor to see the scotopic stimuli. The lowest vision of any participant was 48 letters, equivalent to a logarithm of the minimum angle of resolution score of 0.7. Specifically, in the context of RCD and visual acuity worse than 0.7, the logarithm of the minimum angle of resolution did not allow for meaningful testing of sensitivity given the limited dynamic range of the S-MAIA device. The technique is, therefore, most suitable for either early or intermediate RCD, or in the presence of relatively preserved central vision, or for patients with expected increase in localized function after a therapeutic intervention. FST constitutes an important supplement, especially for patients with low vision and for the assessment of global treatment effects. The two techniques cannot be used interchangeably owing to underlying areas of the retina reflected by the response

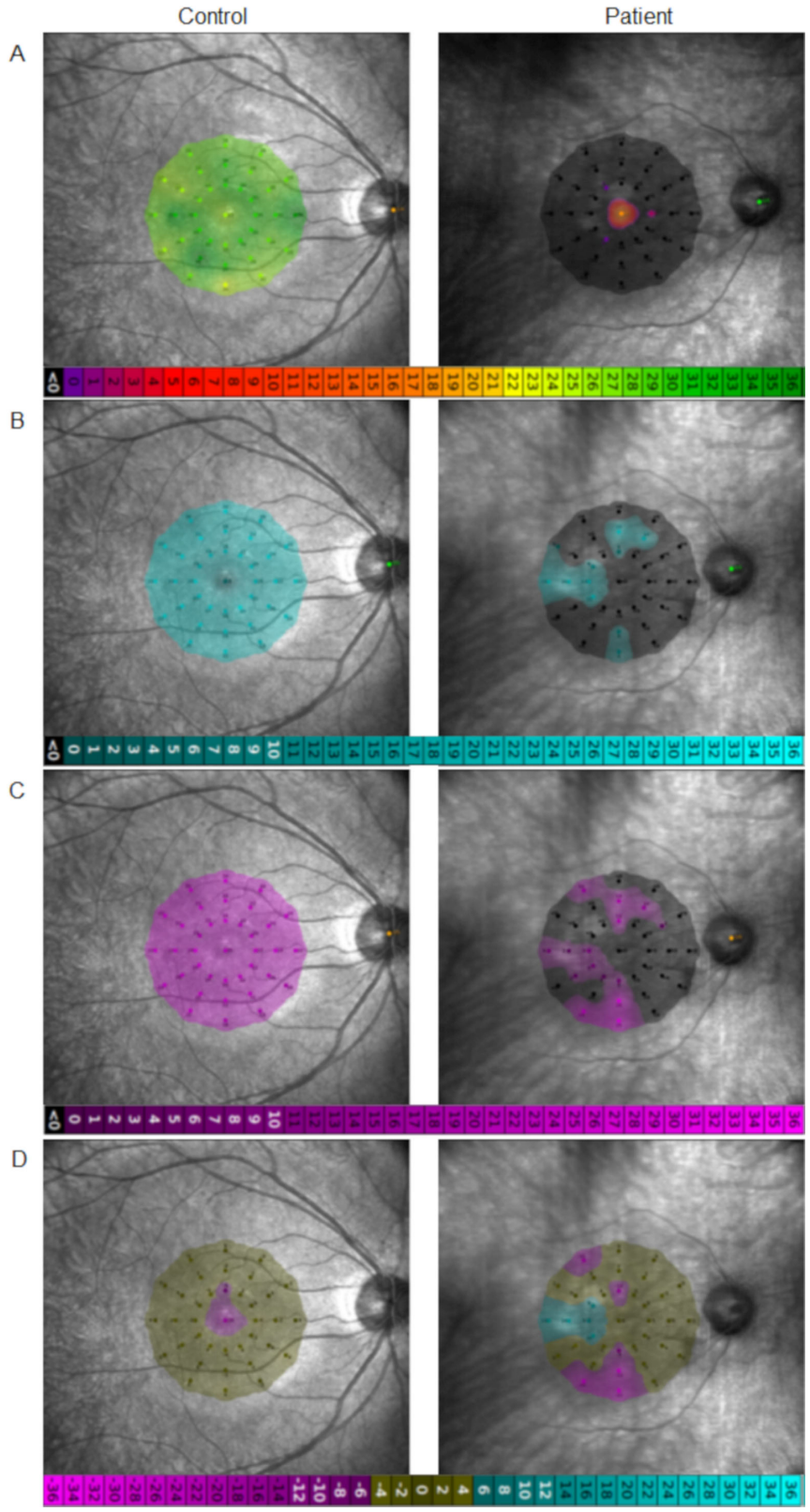

Figure 5. Example results from the S-MAIA for an exemplary control and patient eye. Plots are outputs from the S-MAIA so the scales and color schemes reflect those of the manufacturer. The control group is represented on the left and patient group is represented on the right. (A) Mesopic microperimetry. (B) Scotopic cyan testing (505 nm). (C) Scotopic red testing (627 nm). (D) Difference between cyan and red. Scales for A, B, and D are 0 to 36 dB. Scale for D is −36 to +36 dB.

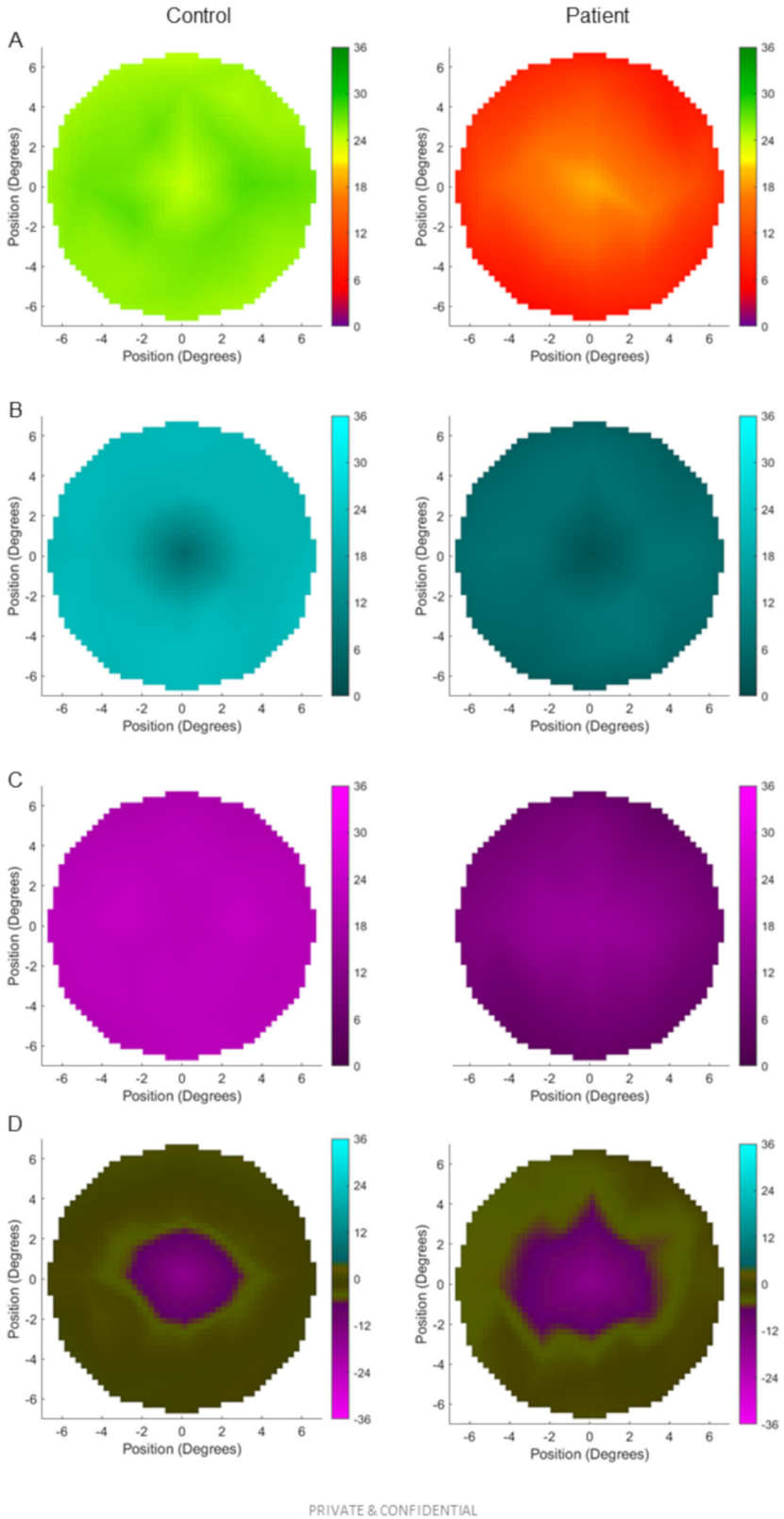

Figure 6. Pointwise averages represented as microperimetry spatial maps. The color bar represents the threshold color scale (dB) for each plot. Control group represented on the left and patient group represented on the right. (A) Mesopic microperimetry. (B) Scotopic cyan testing (505 nm). (C) Scotopic red testing (627 nm). (D) Difference between cyan and red.

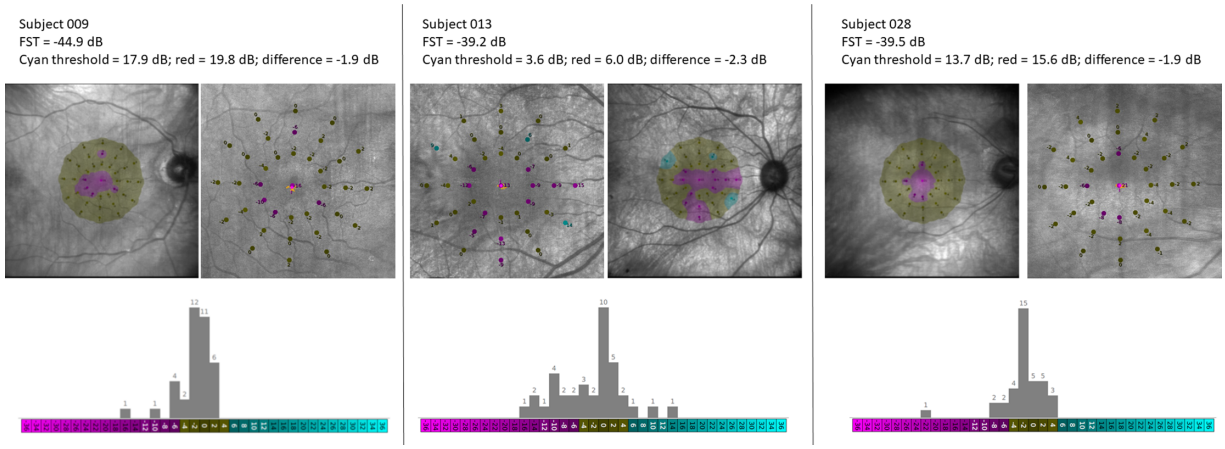

Figure 7. Examples of Ushers phenotypes from subjects in the study, with similar FST results but varying spatial patterns of thresholds for responses to cyan and res stimuli with associated difference maps shown. Both the interpolated and pointwise maps are displayed, along with the histogram of the range of points along the difference axis.

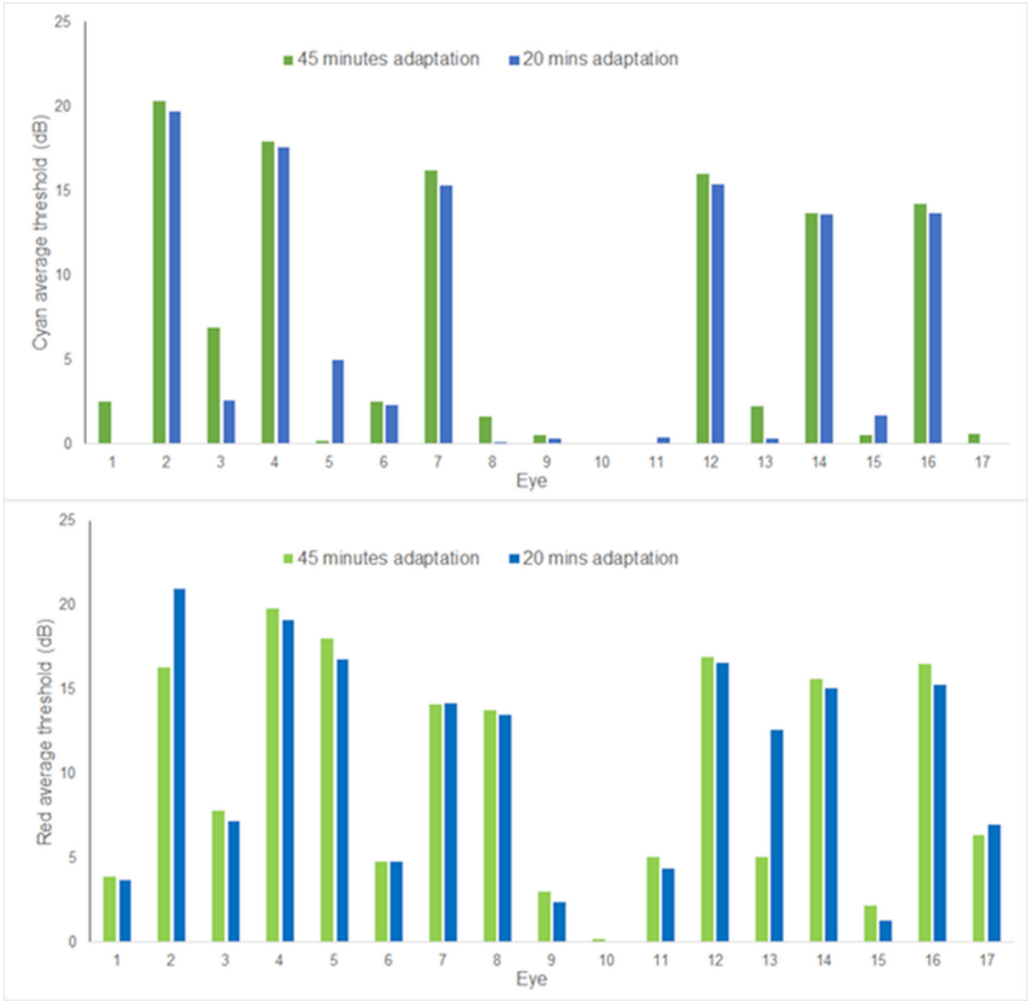

Figure 8. Threshold measurements for each eye with cyan (top) and red (bottom stimuli comparing 45 minutes dark adaptation green bar) versus 20 minutes dark adaptation (blue bar).

and differential intrusion of residual cone sensitivity. This can clearly be seen in the phenotypic exploration in Figure 7 as all eyes have a similar FST. The FST is unable to predict the spatial pattern or thresholds that are revealed on the S-MAIA. Following on from this proof of concept work, several of the authors are undertaking detailed phenotyping work in several genetic conditions.

The optimal adaptation has not been investigated previously in disease. A time period of 20 minutes seems to be sufficient for adequate scotopic threshold determination, with 45 minutes only providing a small margin of benefit to lower vision patients in the cyan range. We, therefore, propose a time period of 20 minutes of dark adaptation before testing. Since the initial submission of this article, another study investigating the adaptation time for scotopic microperimetry also proposes 20 minutes as the optimal time.[37] This decreased time will significantly decrease the burden on patients and increase the feasibility of conducting S-MAIA in a clinical environment.

All microperimetry assessments and FST were able to differentiate between the RCD and control groups. The scotopic provided information about the balance of rods and cones over the central 12° using a 37 stimuli grid. This strategy provided additional valuable information not provided by the FST. Interestingly, few patients showed a negative skew in the difference results. This finding may reflect an altered balance in the rod and cone function in disease as a result of retinal remodelling.[38] Examining S-MAIA in different types of genetic mutations may provide further information about how rod and cone loss occurs in these circumstances and after therapy. We did not have enough patients with confirmed mutations to perform this analysis. However, this proof-of-concept study shows the suitability of scotopic microperimetry in patients with RCD and relatively preserved central vision. S-MAIA can be used as a clinical trial outcome measure, especially in early disease. It can also be used to study patterns of disease degenerations.

Acknowledgments

Funded by the National Institute for Health Research (NIHR) [Clinical Doctoral Research Fellowship CA-CDRF-2016-02-002 for Jasleen K. Jolly]. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health and Social Care. The sponsor and funding organization had no role in the design or conduct of this research.

Disclosure: **J.K. Jolly**, None; **A. Nanda**, None; **T.M.W. Buckley**, None; **M. Pfau**, None; **H. Bridge**, None; **R.E. MacLaren**, None

References

1. Xue K, Jolly JK, Barnard AR, et al. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia. *Nat Med*. 2018;24(10):1507–1512, doi:10.1038/s41591-018-0185-5.
2. Cehajic-Kapetanovic J, Xue K, Martinez-Fernandez de la Camara C, et al. Initial results from a first-in-human gene therapy trial on X-linked retinitis pigmentosa caused by mutations in RPGR. *Nat Med*. 2020;26(3):354–359, doi:10.1038/s41591-020-0763-1.
3. Roman AJ, Cideciyan A V, Wu V, Garafalo A V, Jacobson SG. Full-field stimulus testing: role in the clinic and as an outcome measure in clinical trials of severe childhood retinal disease. *Prog Retin Eye Res*. 2022;87:101000. Published online 2021:101000, doi:10.1016/j.preteyeres.2021.101000.
4. Roman AJ, Schwartz SB, Aleman TS, et al. Quantifying rod photoreceptor-mediated vision in retinal degenerations: dark-adapted thresholds as outcome measures. *Exp Eye Res*. 2005;80(2):259–272, doi:10.1016/j.exer.2004.09.008.
5. Dimopoulos IS, Freund PR, Knowles JA, MacDonald IM. The natural history of full-field stimulus threshold decline in choroideremia. *Retina*. 2018;38(9):1731–1742, doi:10.1097/IAE.0000000000001764.
6. Jolly JK, Bridge H, MacLaren RE. Outcome measures used in ocular gene therapy trials: a scoping review of current practice. *Front Pharmacol*. 2019;10:1076, doi:10.3389/fphar.2019.01076.
7. Simunovic MP, Moore AT, MacLaren RE. Selective automated perimetry under photopic, mesopic, and scotopic conditions: detection mechanisms and testing strategies. *Transl Vis Sci Technol*. 2016;5(3):1–13, doi:10.1167/tvst.5.3.10.
8. Jacobson SG, Voigt WJ, Parel JM, et al. Automated light- and dark- adapted perimetry for evaluating retinitis pigmentosa. *Ophthalmology*. 1986;93(12):1604–1611, doi:10.1016/S0161-6420(86)33522-X.
9. Massof RW, Finkelstein D. Rod sensitivity relative to cone sensitivity in retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 1979;18(3):263–272.
10. Fraser RG, Tan R, Ayton LN, Caruso E, Guymer RH, Luu CD. Assessment of retinotopic rod

photoreceptor function using a dark-adapted chromatic perimeter in intermediate age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2016;57(13):5436–5442, doi:10.1167/iovs.16-19295.
11. Owsley C, Jackson GR, Cideciyan A V., et al. Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000;41(1):267–273.
12. Birch DG, Wen Y, Locke K, Hood DC. Rod sensitivity, cone sensitivity, and photoreceptor layer thickness in retinal degenerative diseases. *Invest Ophthalmol Vis Sci*. 2011;52(10):7141–7147, doi:10.1167/iovs.11-7509.
13. Steinmetz RL, Haimovici R, Jubb C, Fitzke FW, Bird AC. Symptomatic abnormalities of dark adaptation in patients with age-related Bruch's membrane change. *Br J Ophthalmol*. 1993;77(9):549, doi:10.1136/bjo.77.9.549.
14. Flynn OJ, Cukras CA, Jeffrey BG. Characterization of rod function phenotypes across a range of age-related macular degeneration severities and subretinal drusenoid deposits. *Invest Ophthalmol Vis Sci*. 2018;59(6):2411–2421, doi:10.1167/iovs.17-22874.
15. McGuigan DB, Roman AJ, Cideciyan A V., et al. Automated light- and dark-adapted perimetry for evaluating retinitis pigmentosa: filling a need to accommodate multicenter clinical trials. *Invest Ophthalmol Vis Sci*. 2016;57(7):3118–3128, doi:10.1167/iovs.16-19302.
16. Bennett LD, Klein M, Locke KG, Kiser K, Birch DG. Dark-adapted chromatic perimetry for measuring rod visual fields in patients with retinitis pigmentosa. *Transl Vis Sci Technol*. 2017;6(4):15, doi:10.1167/tvst.6.4.15.
17. Pfau M, Jolly JK, Wu Z, et al. Fundus-controlled perimetry (microperimetry): application as outcome measure in clinical trials. *Prog Retin Eye Res*. 2021;81:100893. Published online 2020:100907, doi:10.1016/j.preteyeres.2020.100907.
18. Taylor LJ, Josan AS, Pfau M, Simunovic MP, Jolly JK. Scotopic microperimetry: evolution, applications and future directions. *Clin Exp Optom*. 2022;105(8):793–800. Published online 2022, doi:10.1080/08164622.2021.2023477.
19. Pfau M, Lindner M, Fleckenstein M, et al. Test-retest reliability of scotopic and mesopic fundus-controlled perimetry using a modified MAIA (macular integrity assessment) in normal eyes. *Ophthalmologica*. 2017;237(1):42–54, doi:10.1159/000453079.
20. Pfau M, Lindner M, Steinberg JS, et al. Visual field indices and patterns of visual field deficits in mesopic and dark-adapted two-colour fundus-controlled perimetry in macular diseases. *Br J Ophthalmol*. 2018;102(8):1054–1059. Published online 2017, doi:10.1136/bjophthalmol-2017-311012.
21. Heeren TFC, Tzaridis S, Bonelli R, et al. Dark-adapted two-color fundus-controlled perimetry in macular telangiectasia type 2. *Invest Ophthalmol Vis Sci*. 2019;60(5):1760–1767, doi:10.1167/iovs.18-25360.
22. Pfau M, von der Emde L, Dysli C, et al. Light sensitivity within areas of geographic atrophy secondary to age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2019;60(12):3992–4001, doi:10.1167/iovs.19-27178.
23. von der Emde L, Pfau M, Thiele S, et al. Mesopic and dark-adapted two-color fundus-controlled perimetry in choroidal neovascularization secondary to age-related macular degeneration. *Transl Vis Sci Technol*. 2019;8(1):7, doi:10.1167/tvst.8.1.7.
24. Hess K, Gliem M, Charbel Issa P, et al. Mesopic and scotopic light sensitivity and its microstructural correlates in pseudoxanthoma elasticum. *JAMA Ophthalmol*. 2020;138(12):1272–1279, doi:10.1001/jamaophthalmol.2020.4335.
25. Naska KTK, Hogg R, Morales MU, Amoaku WMK. Impact of dark adaptation time on the Scotopic microperimeter S-MAIA. *Invest Ophthalmol Vis Sci*. 2018;59(9):1273.
26. Robson AG, Nilsson J, Li S, et al. ISCEV guide to visual electrodiagnostic procedures. *Doc Ophthalmol*. 2018;136(1):1–26, doi:10.1007/s10633-017-9621-y.
27. Moore AT, Fitzke FW, Kemp CM, et al. Abnormal dark adaptation kinetics in autosomal dominant sector retinitis pigmentosa due to rod opsin mutation. *Br J Ophthalmol*. 1992;76(8):465–469, doi:10.1136/bjo.76.8.465.
28. Han RC, Gray JM, Han J, Maclaren RE, Jolly JK. Optimisation of dark adaptation time required for mesopic microperimetry. *Br J Ophthalmol*. 2019;103(8):1092–1098. Published online 2018:1–7, doi:10.1136/bjophthalmol-2018-312253.
29. Curcio CA, Allen KA, Sloan KR, et al. Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. *J Comp Neurol*. 1991;312(4):610–624, doi:10.1002/cne.903120411.
30. RConnie Han, JKaur Jolly, Xue K, MacLaren RE. Effects of pupil dilation on MAIA microperimetry. *Clin Exp Ophthalmol*. 2017;45(5):489–495, doi:10.1111/ceo.12907.
31. Chen FK, Patel PJ, Xing W, et al. Test-retest variability of microperimetry using the Nidek MP1 in patients with macular disease. *Invest Ophthal-*

mol Vis Sci. 2009;50(7):3464–3472, doi:10.1167/iovs.08-2926.

32. Shpak M, Kärhä P, Porrovecchio G, Smid M, Ikonen E. Luminance meter for photopic and scotopic measurements in the mesopic range. *Meas Sci Technol*. 2014;25(9):09501, doi:10.1088/0957-0233/25/9/095001.
33. Roman AJ, Cideciyan AV, Wu V, Garafalo AV, Jacobson SG. Full-field stimulus testing: role in the clinic and as an outcome measure in clinical trials of severe childhood retinal disease. *Prog Retin Eye Res*. 2022;87:101000, doi:10.1016/j.preteyeres.2021.101000
34. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol*. 1990;292:497–523.
35. Wu Z, Ayton LN, Guymer RH, Luu CD. Intrasession test-retest variability of microperimetry in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2013;54(12):7378–7385, doi:10.1167/iovs.13-12617.
36. Welker SG, Pfau M, Heinemann M, Schmitz-Valckenberg S, Holz FG, Finger RP. Retest reliability of mesopic and dark-adapted microperimetry in patients with intermediate age-related macular degeneration and age-matched controls. *Invest Ophthalmol Vis Sci*. 2018;59(4):AMD152–AMD159, doi:10.1167/iovs.18-23878.
37. Montesano G, Naska TK, Higgins BE, Wright DM, Hogg RE, Crabb DP. Determinants of test variability in scotopic microperimetry: effects of dark adaptation and test indices. *Transl Vis Sci Technol*. 2021;10(1):1–11, doi:10.1167/tvst.10.1.26.
38. Marc RE, Jones BW. Retinal remodeling in inherited photoreceptor degenerations. *Mol Neurobiol*. 2003;28(2):139–147, doi:10.1385/MN:28:2:139.