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Longitudinal Changes in Scotopic and Mesopic Macular Function as Assessed with Microperimetry in Patients with Stargardt Disease: SMART Study Report No. 2

Xiangrong Kong*[1-4], Mohamed Ibrahim-Ahmed* [5], Millena G. Bittencourt[1], Rupert W. Strauss[6-8], David G. Birch[9], Artur V. Cideciyan[10], Ann-Margaret Ervin [1,3], Alexander Ho, [11#], Janet S Sunness[12], Isabelle S. Audo[13], Michel Michaelides[6], Eberhart Zrenner,[14], SriniVas Sadda[11], Michael S. Ip[11], Sheila West[1], Hendrik P.N. Scholl[1,15,16], for the SMART Study Group

Authors Affiliations
[1.] Wilmer Eye Institute at the Johns Hopkins University, Baltimore, MD, USA.
[2.] Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.
[3.] Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.
[4.] Department of Health Behavior and Society, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.
[5.] Ocular Imaging Research and Reading Center, Sunnyvale, CA, USA.
[6.] Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, United Kingdom.
[7.] Department of Ophthalmology, Kepler University Clinic, Linz, Linz, Austria.
[8.] Department of Ophthalmology, Medical University of Graz, Graz, Austria.
[9.] Retina Foundation of the Southwest, Dallas, TX, USA.
[10.] Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA, USA.
[11.] Doheny Image Reading Center, Los Angeles, CA, USA.
[12.] Greater Baltimore Medical Center, Baltimore, MD, USA.
[13.] CHNO des Quinze-Vingts, DHU Sight Restore, INSERM-DHOS CIC 1423, Sorbonne Universités, UPMC Univ Paris 06, INSERM, CNRS, Institut de la Vision, Paris, France.
[14.] Universitäts-Augenklinik, University of Tübingen, Tübingen, Germany
[15.] Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland.
[16.] Department of Ophthalmology, University of Basel, Basel, Switzerland.
*:equal contributions. [#]: current affiliation: Labcorp Drug Development.

Short Title:
Scotopic and Mesopic Macular Functions in Stargardt Disease

Corresponding Authors:
Xiangrong Kong
Woods 155B
The Wilmer Eye Institute
600 N. Wolfe Street
Baltimore, MD 21287
USA
Telephone: 410-614-4087
xkong4@jhu.edu

Hendrik P. N. Scholl,
Institute of Molecular and Clinical Ophthalmology Basel (IOB)
Mittlere Strasse 91, CH-4031 Basel, Switzerland
Telephone (Office): +41 61 265 86 51
E-mail: hendrik.scholl@iob.ch

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Abstract

Purpose: To estimate and compare cross-sectional scotopic versus mesopic macular sensitivity losses measured by microperimetry, and to report and compare the longitudinal rates of scotopic and mesopic macular sensitivity losses in *ABCA4* gene associated Stargardt Disease (STGD1).

Design: Multicenter prospective cohort study.

Methods: Participants: 127 molecular confirmed STGD1 patients enrolled from 6 centers in the USA and Europe and followed every 6 months for up to 2 years.

Observation Procedures: The Nidek MP-1S device was used to measure macular sensitivities of the central 20° under mesopic and scotopic conditions. The mean deviations (MD) from normal for mesopic macular sensitivity for the fovea (within 2° eccentricity) and extrafovea (4°-10° eccentricity), and the MD for scotopic sensitivity for the extrafovea were calculated. Linear mixed effects models were used to estimate mesopic and scotopic changes.

Main Outcome Measures: Baseline mesopic mean deviation (mMD) and scotopic MD (sMD) and rates of longitudinal changes in the mMDs and sMD.

Results: At baseline, all eyes had larger sMD, and the difference between extrafoveal sMD and mMD was 10.7 dB (p<.001). Longitudinally, all eyes showed a statistically significant worsening trend: the rates of foveal mMD and extrafoveal mMD and sMD changes were 0.72 (95%CI: 0.37 to 1.07), 0.86 (95%CI: 0.58 to 1.14) and 1.12 (95%CI: 0.66 to 1.57) dB/year, respectively.

Conclusions: In STGD1, in extrafovea, loss of scotopic macular function preceded and was faster than the loss of mesopic macular function. Scotopic and mesopic

macular sensitivities using microperimetry provide alternative visual function outcomes for STGD1 treatment trials.

Introduction

Stargardt disease type 1 (STGD1) is the most prevalent juvenile macular dystrophy with an estimated population prevalence of 1/6,500 in the US, and can affect both children and adults[1-4]. It is caused by variants in the *ABCA4* gene, and primarily inherited as an autosomal recessive trait[3]. The disease exhibits great genotypic and phenotypic heterogeneity, but the typical pathology involves atrophy of the para-foveal and/or foveal regions of the macula, with expansion to the peripheral retina over time[3]. Clinically, patients experience a gradual loss of central vision, and may reach legal blindness over decades[5,6].

The *ABCA4* gene is expressed in both rod and cone photoreceptors[7-9], and both cone and rod functions are affected in STGD1 as a consequence of pathogenic variants in *ABCA4*. A study at the photoreceptor level using Adaptive Optics Scanning Light Ophthalmoscopy (AOSLO) technology suggested that at the early stage loss of cone photoreceptors focused in the fovea whereas peripheral loss focused on rod photoreceptors[10]. Understanding clinically the temporal and spatial loss of different photoreceptor types will facilitate a better understanding of disease pathophysiology and can inform choices of clinical measurements based on the specific pathways targeted by therapeutic interventions.

Microperimetry (MP), an automated fundus perimetry with live eye tracking, has become a useful clinical tool to monitor retinal disease progression [11]. The quantitative

macular sensitivity measurements generated from MP also provide potential visual function outcome measures for STGD1 treatment trials. A few studies have reported macular sensitivity loss in STGD1 under mesopic conditions using cross-sectional and longitudinal MP data[12-19], but there is little available data describing scotopic macular function loss in STGD1[20 21].

The prospective “Progression of Atrophy Secondary to Stargardt Disease” (ProgStar) study (ClinicalTrials.gov ID: NCT01977846) is a multicenter natural history study designed to assess visual function and retinal morphological changes in STGD1 and to help identify appropriate outcome measures for STGD1 treatment trials[22]. Its ancillary study, the “Scotopic Microperimetric Assessment of Rod Function in Stargardt Disease” (SMART) study, focused on assessing macular function loss under scotopic conditions in STGD1[23].

To better understand macular function loss in STGD1 natural history and to assess the potential of macular sensitivity measurements as primary outcome measures for STGD1 trials, the current analysis used the longitudinal data of ProgStar participants enrolled in the SMART study and aimed to compare cross-sectional scotopic versus mesopic macular sensitivity loss, to report the longitudinal rate of scotopic macular sensitivity loss and to compare it to the rate of mesopic macular sensitivity loss.

Methods

The study designs of the ProgStar and the SMART studies were reported previously[22,23]. In brief, the international ProgStar study enrolled 259 STGD1 patients from 9 clinical centers in the US, United Kingdom, France and Germany during 2014-

15. Eligibility of participants and inclusion criteria of study eyes particularly relevant here included: age≥ 6 years and having 2 pathogenic mutations in the ABCA4 gene, or having 1 pathogenic mutation in the ABCA4 gene together with a typical STGD1 phenotype; and eyes having a best corrected visual acuity (BCVA) of 20 or more Early Treatment Diabetic Retinopathy Study (ETDRS) letters (i.e., 20/400 Snellen equivalent or better), and having at least 1 well-demarcated area of atrophy on fundus autofluorescence (FAF) imaging with a diameter of 300 μm or more and the sum of all lesions of 12 mm^2 or less (further inclusion/exclusion criteria are described in ProgStar report no. 1 [22]). All participants provided written informed consent before enrollment in the study. ProgStar participants were followed every 6 months for 2 years. At each study visit, participants underwent a detailed ophthalmic exam, BCVA testing using the ETDRS protocol [24], and mesopic MP testing. FAF and spectral domain ocular coherence tomography (SD-OCT) images were also obtained.

After the ProgStar study started enrollment, the SMART study was initiated and 6 ProgStar sites participated. Informed consents were obtained for ProgStar participants to undergo scotopic MP testing during their remaining ProgStar visits. Only one eye was selected to undergo scotopic testing. The selection was determined by the site principal investigators and they were recommended by the study protocol to select the eye with the smaller lesion from FAF imaging, better BCVA, and/or better fixation[23]. Mesopic and scotopic MP tests were repeated at each subsequent available ProgStar follow-up visit using the follow-up function of the MP device.

Nidek MP-1S (Nidek Technologies, Inc., Gamagori, Japan) and the associated NAVIS Software (v.1.7.7 or higher) (Nidek Technologies S.R.L.) were used for both

mesopic and scotopic MP testing. Procedures for the mesopic testing and fixation assessment were described previously [19,25]. Briefly, under dim room lighting, mesopic macular sensitivity was tested at 68 retinal locations using a pattern comparable to the Humphrey 10-2 protocol (**Error! Reference source not found.** Left). A white stimulus (0.43 degree diameter; comparable to Goldmann III) was used with a duration of 200 ms and on a dim white background (1.27 candela (cd)/m^2). Both cones and rods should contribute to the sensitivity response under such testing conditions. The maximum stimulus luminance in MP-1S was 127 cd/m^2 [26].

Under the SMART study protocol, testing under scotopic conditions was added where macular response was predominantly driven by rod photoreceptors in normal eyes. The scotopic testing was performed in a fully darkened room. Sensitivity was tested at 40 retinal locations distributed in a region that extended 4-10 degrees from the foveal center in a custom pattern shown in **Error! Reference source not found.** Right. Before scotopic testing, the eye selected for SMART was occluded for dark adaptation using a double pad and eye patch for at least 30 minutes. To maintain the dark-adapted state, the test background was changed to dim red, and a short-pass (<500 nm, blue) filter (NT52532, Edmund Optics, Barrington, NJ) was inserted into the light path. The dynamic range of the test stimuli was tuned to the typical range of the rod threshold by inserting neutral density (ND) filters. Considering the already impaired visual functions in STGD1 patients, the SMART protocol requested a 1.0-log unit ND filter (1.0ND) which attenuated the luminance of the test stimuli by a factor of 10, although the commonly used 2.0ND filter was also provided. The ND filter used was recorded in the data collection form.

In both the scotopic and the mesopic tests, the SD-OCT image was used to center the test pattern on the anatomical fovea as accurately as possible by the photographer. The sensitivity at each retinal location was determined using the 4-2 threshold strategy. A sensitivity value of 0 dB corresponded to the brightest stimulus applied and a 20 dB value corresponded to the dimmest stimulus. To ensure optimal tracking of the fundus during the testing, participants were instructed to fixate on a fixation target throughout testing (a red cross for the mesopic test and a “white” circle that appeared blue due to the inserted blue filter for the scotopic test). All examinations were performed monocularly with the contralateral eye patched. The mesopic test always preceded the scotopic test for SMART study eyes, and both MP tests always preceded FAF imaging.

The pattern placement on the fovea was categorized as “adequate”, “fair”, “poor”, or “cannot grade” by the study central reading center. When the center of the grid was ≤1° from the anatomical fovea this was graded as “adequate”. Distances from 1° to less than 2° were “fair”. “Poor” pattern placement meant that the grid was improperly placed at a distance ≥2° and these test results were excluded from subsequent analyses.

Macular Sensitivity

The dB output from the MP-1 is a unit relative to the maximum luminance of the MP test which was different between mesopic and scotopic conditions. The thresholds under mesopic and scotopic conditions in normal eyes are also different. Therefore, direct comparison of the numerical output from the above mesopic and scotopic MP tests would not be meaningful. For mesopic response, the dB output from the MP-1 (denoted as X_m) was converted to the mean deviation (MD) from the maximum

measurable normal mesopic sensitivity, that is, the mesopic MD was calculated as (20-X_m) [27]. For scotopic response, the dB value (denoted as X_s) was converted to the MD from the maximum measurable normal scotopic mean sensitivity under the scotopic testing condition using the 2ND filter (i.e. 20dB)[28], that is, the scotopic MD was calculated as (20- X_s) for eyes where the 2ND filter was used and as [20-(X_s-10)] for eyes where the 1ND filter was used.

The scotopic MP test pattern covered the extrafoveal region extending 4°-10° eccentricity. To compare mesopic and scotopic macular function losses in this same region (Figure 1 Left), the mesopic MD (mMD) of the 64 test loci in this region was calculated (Figure 1 Right), and the scotopic MD (sMD) was calculated from the 40 test loci. Fovea mMD was also calculated using the 4 loci within 2° eccentricity from the mesopic MP test.

Statistical Analyses

At the first visit for the SMART study, participants' corresponding mesopic MP test results were abstracted from the ProgStar study database. Paired t-test was used to compare the extrafoveal mMD and sMD, and Pearson correlation coefficient was estimated for the extrafoveal mMD and sMD. A linear regression model was also used to assess whether the difference between extrafoveal mMD and sMD was associated with the level of scotopic function loss (i.e. sMD). In addition, the foveal mMD and extrafoveal sMDs were estimated for the subgroups of eyes that had "approximately normal" extrafoveal mesopic sensitivity and of eyes that had "approximately normal" foveal mesopic sensitivity (operationally defined as mesopic mean sensitivity from MP1-S ≥12dB, i.e. mMD<8 dB [29]).

Longitudinally, the rate of change of each MP parameter was estimated using a linear mixed effect model (LMEM), where the mean of the parameter was modeled as a linear function of time since the first visit, with the intercept and slope parameter assumed to be random effects following normal distributions. Such modeling implicitly accounted for the correlation from repeated measurements. To compare between the rates of changes of extrafoveal sMD and mMD, the LMEM was extended to include an indicator of MP test type and its interaction with time. The coefficient of the interaction term quantifies the difference in the rates of changes of extrafoveal MDs between the scotopic MP and mesopic MP tests.

All analyses were conducted in SAS 9.4, and two-sided p-values from Wald-tests were reported. The model fit for LMEMs was inspected visually and based on plots of scaled residuals [30].

Results

Participants Disposition and Demographics

The SMART study enrolled 130 participants (eyes) and scotopic MP was tested for a total of 497 eye-visits. Among these eye-visits, 4 scotopic tests (0.8%) and 6 mesopic MP tests (1.2%) were deemed ungradable by the reading center, and 41 scotopic tests (8.3%) and 60 mesopic tests (12.1%) had poor pattern placement. Among the 442 eye-visits with scotopic MP of adequate or fair pattern placement, 401 eye-visits had mesopic MP tests of adequate or fair pattern placement. These 401 eye-visits were from 127 of the SMART participants (97.7%), and their baseline data (i.e., their first SMART visit) were used to compare between mesopic and scotopic sensitivity impairments cross-sectionally. There were 116 participants for whom data for at least 2

visits were available for the longitudinal data analysis. Seventy-three eyes contributed data at the 6-months follow-up, 89, 83, and 29 eyes contributed data at the 12, 18 and 24-months follow-ups, respectively.

Table 1 summarizes the baseline characteristics for the 127 SMART participants: 52% were female, 83% were white, mean age was 34.5 (standard deviation [SD]=15.1) years, and mean BCVA was 52 ETDRS letters (20/91 Snellen equivalent) (SD=19 letters). The mean age at symptom onset was 24.7 (SD=14.5) years and the mean duration of symptoms was 10.1 years (SD=7.0); with 41.7% of the cohort reporting symptom onset at an age ≤18 years (Table 1).

Mean Deviations (MD) at the Baseline Visit

At SMART baseline (Table 1), the median mesopic MD (mMD) of the overall central 20° test field was 8.4 dB (interquartile range [IQR] 4.8 to 10.8). The median mMD for the foveal region was 16.8 (IQR 15.0 to 20.0) dB. The median mMD and scotopic MD (sMD) for the extrafoveal region was 7.9 dB (IQR 4.2 to 10.3) and 18.6 dB (IQR 14.9 to 21.8), respectively. Figure 2A shows the cross-sectional distribution of sMD and mMD for the extrafovea. The mean difference between extrofoveal sMD and mMD was 10.7 dB (95%CI 10.1 to 11.3, p<.001) (positive [sMD-mMD] value indicates larger scotopic impairment). The correlation coefficient between extrafoveal sMD and mMD was 0.82 (p<.001). All eyes had mMD smaller than sMD, indicating larger impairment of scotopic sensitivity in the extrafoveal region. Figure 2B is the scatterplot showing the difference between extrafoveal scotopic and mesopic MDs as a function of extrafoveal sMD: the positive linear trend had a slope of 0.32 (95%CI: 0.20 to 0.43, p<.001), suggesting that every 1dB larger scotopic sensitivity loss was significantly

associated with 0.32 dB greater difference between mesopic and scotopic sensitivity impairments.

At baseline, there were 81 eyes that had “approximately normal” extrafoveal mMP (MD<8dB). Among these eyes, the median foveal mMD was 19.0 (IQR 11.5 to 20; range 0 to 20) dB; and the median extrafoveal sMD was 15.6 (IQR 13.7 to 17.8, and range 0.6 to 29.1) dB (Table 1).

There were 11 eyes that had “approximately normal” foveal mMP (i.e. sensitivity ≥12dB) (Supplemental table 1). Among these eyes, the median extrafoveal mMD was 1.7 dB (IQR 0.6 to 3.7, range 0 to 9.3); the median extrafoveal sMD was 12.6 dB (IQR 10.4 to 15. 6, range 0.6 to 25.7); and the median difference between extrafoveal sMD and mMD was 11.6 dB (IQR 6.7 to 12.6, range 0.5 to 17.6) dB (sMD-mMD positive indicates larger scotopic impairment).

Longitudinal Changes in Mean Deviations (MD)

Figure 3 shows the longitudinal data of foveal mMD and extrafoveal mMD and sMD of individual eyes. Large within-eye variability across visits for foveal mMD was observed for some eyes (Figure 3A). Nevertheless, the average of all eyes showed a statistically significant worsening trend and the rate of foveal mMD change was 0.72 (95%CI: 0.37 to 1.07) dB/year (Table 2). The rates of extrafoveal mMD and sMD changes were 0.86 (95%CI: 0.58 to 1.14) dB/year and 1.12 (95%CI: 0.66 to 1.57) dB/year (Figure 3 B-C) (Table 2), respectively.

Comparing between the rates of mesopic and scotopic changes, the model using both extrafoveal mMD and sMD data estimated that the difference in the rate of change

of extrafoveal sMD compared to mMD was 0.41 (95%CI: -0.07 to 0.89) dB/year (p=0.10).

For the subgroup of 81 eyes with “approximately normal” extrafoveal mMD at baseline, during follow-up, the rates of foveal mMD and extrafoveal mMD and sMD changes were 0.96 (95%CI: 0.47 to 1.46), 0.96 (95%CI: 0.61 to 1.32) dB/year, and 1.31 (95%CI: 0.74 to 1.89) dB/year, respectively (Table 2).

For the subgroup of 11 eyes with “approximately normal” foveal mMD at baseline, during follow-up, the rates of foveal mMD and extrafoveal mMD and sMD changes were 2.98 (95%CI: --0.21, 6.18) dB/year, 1.20 (95%CI: -0.06 to 2.46) dB/year, and 1.58 (95%CI: -0.84 to 4.00) dB/year, respectively (Table 2). This subgroup also included 2 eyes that had both extrafoveal mesopic and scotopic sensitivity responses that were normal at baseline (MDs~0dB). Their baseline and last follow-up data are shown in Table 3. At baseline Eye 1 had nearly normal foveal mMD (2.0 dB), and normal extrafoveal mMD (0.94 dB)) and sMD (1.53 dB). Its BCVA was 20/110 and had a lesion involving the fovea shown in FAF imaging (area of decreased autofluorescence [DAF] =2.19mm^2) (Figure 4A left). At the last visit (18 months later), its extrafovea mMD was normal, but its extrafovea sMD declined to 10.2 dB loss and foveal mMD declined to 10 dB associated with the area of DAF slightly increased to 2.25 mm^2 (Figure 4A right). Eye 2 at baseline had normal foveal and extrafoveal mesopic and scotopic sensitivity responses, but the BCVA was 20/126. A DAF of size 0.57mm^2 involved the foveola (Figure 4B left). At the last visit (18 months later) (Figure 4B right), the foveal mMD, extrafoveal mMD and sMD increased to 20 dB, 4.41 dB, and 11.28 dB, respectively. The DAF grew to 1.59mm^2.

Discussion

Emerging therapeutic options for STGD1 need to be tested in clinical trials. Identifying appropriate outcome measures that are clinically meaningful and can reflect disease progression in a relatively short time such as 1 or 2 years is important for the design of treatment trials aimed to slow loss of visual functions in STGD1. This led to the ProgStar study the primary aims of which were to estimate the rates of disease progression of retinal structural parameters measured by FAF and SD-OCT and visual functions measured by BCVA and macular sensitivity from microperimetry. The SMART study was further added to estimate scotopic macular function loss and to test the hypothesis that rod function loss was faster than cone loss.

Previous findings of the ProgStar study have repeatedly shown that BCVA decline is slow in STGD1 and the change in BCVA does not show a statistically significant association with growth of area of atrophy measured by FAF.[31-34] The ProgStar and SMART study protocols provide natural history data on functional declines in mesopic and scotopic macular sensitivities (MS) over a follow-up of up to 24 months. The MSs were measured as the mean deviations from the maximally measurable normal using the MP-1S device. The rates of MS declines were statistically significant and clinically relevant, thus mesopic and scotopic MSs were more sensitive to reflect functional change than BCVA in STGD1.

Our data suggest that the loss of scotopic macular function is faster than the loss of mesopic macular function in the extrafovea. Thus, sMD may be a more sensitive outcome measure compared to mMD. However, the scotopic MP testing requires dark adaption of at least 30 minutes and thus imposes more testing burden for patients and

clinical staff. Additionally, because sMD had larger variability than mMDs (Table 1), using sMD may not yield a smaller sample size compared to using mMD when designing a clinical trial. Nevertheless, because the loss of scotopic macular function precedes the loss of mesopic macular response in the extrafovea, sMD provides a candidate functional outcome measure that allows trials to enroll earlier staged STGD1 patients where mesopic macular function in the extrafovea has yet to show impairment. For such early stage patients, the foveal mesopic macular function may also provide a sensible functional outcome measure. Scotopic MS also provides a direct functional outcome if the immediate therapeutic target is to preserve rod function.

At the SMART baseline, in all eyes, the scotopic macular function loss was greater than the mesopic function loss in the extrafovea. This suggests that in extrafovea, scotopic function loss could have started prior to mesopic function loss, and/or that loss of scotopic function was faster than the loss of mesopic function. The observation that at baseline, the greater the loss of scotopic function the larger discrepancy between extrafoveal scotopic and mesopic losses (Figure 2B) implies that scotopic function loss must have occurred at a faster speed than the loss of mesopic function. This is further supported by the rates of extrafoveal sMD and mMD changes estimated from the longitudinal data (sMD 1.12 dB/year vs. mMD 0.86 dB/year). Statistically these rates of extrafoveal scotopic and mesopic function losses were not significantly different ($p=0.10$), but the parameter estimates themselves are informative especially in observational studies[35]. The lack of statistical significance could be due to an insufficient sample size. The length of follow-up time (≤2 years) was also limited and this length of period may be insufficient to statistically differentiate the trajectories of

scotopic and mesopic function losses given the sample size, especially that only 29 eyes contributed data over 24-months of follow-up.

There is also evidence that scotopic function loss precedes mesopic function loss in the extrafovea. In the subgroup of 81 eyes with approximately normal extrafoveal mesopic response at baseline, concurrently there were already profound losses of scotopic MP response (median sMD=15.6 dB). Moreover, despite such losses at baseline, longitudinally, the rate of scotopic MP function loss was greater than the rate of extrafoveal mesopic MP loss.

STGD1 is predominantly a central disease, thus expectedly only a small proportion of eyes had approximately normal fovea macular function (N=11). This group of eyes, especially those with no or mildly impaired acuity (Supplemental table 1), may include the clinical phenotype of fovea sparing[36]. In this group, there was minimal to no loss of extrafoveal mesopic function (median mMD=1.7 dB), but the concurrent scotopic function loss was much larger (median sMD=12.6 dB). This may suggest that in certain STGD1 genotypes, moderate to large loss in extrafoveal scotopic response may be present while fovea mesopic function remains relatively preserved. Longitudinally, however, this group of eyes in average showed a fast decline in foveal mesopic function. This group also included the 2 eyes that had normal extrafoveal scotopic and mesopic responses at baseline (Table 2). They both had poor BCVA. Over an 18 months period, both eyes had a large loss of foveal mesopic function as well as extrafoveal scotopic function, whereas the extrafoveal mesopic function loss was much smaller.

One of the SMART study's major goals was to test the hypothesis that rod function loss was faster than that of cones. In normal eyes, the extrafoveal scotopic MP response would be primarily mediated by rods, and both rods and cones would mediate sensitivity response under the mesopic condition though the exact contribution from each photoreceptor system is difficult to determine[37]. The rod mediated scotopic response however can be altered in diseased retinas[28], and the exact contribution due to rod mediation versus cone mediation cannot be deduced without comparison of dark-adapted responses under two colors of perimetry[18,20,38,39]. Therefore, a direct comparison of rod versus cone function loss could not be made with Progstar and SMART data. Nevertheless, the cross-sectional comparisons at baseline showed significantly different levels of deviations of scotopic compared to mesopic MP tests. This suggests that the sensitivity responses under the scotopic and mesopic conditions were mediated by different combinations of photoreceptors.

A previous study of 66 STGD1 patients reported a rate of rod function loss of 1.1 dB/year using two-color dark adapted perimetry and a rate of cone function loss of 0.45 dB/year using light-adapted perimetry with an orange (600 nm) target.[20] Our longitudinal data estimated a rate of sMD change of 1.12dB/year and a rate of mMD change of 0.86 dB/year (Table 2). The sMD rate is comparable to the previously reported 1.1 dB/year of rod function loss, and the mMD rate is in between the previously reported rates of rod and cone function losses. Hence it is reasonable to assume that in the SMART study more rods were mediating the scotopic response and a combination of cones and rods were mediating the mesopic response. It thus can be inferred that, although direct quantitative comparisons of rod and cone function losses cannot not be

made with SMART and ProgStar data, our results for the extrafovea sensitivity responses support the conclusion that rod function loss precedes and declines faster than cone function loss in STGD1. Our results obtained in the fovea and extrafovea also support the observation of “cone loss predominates centrally and rod loss increases peripherally” based on AOSLO imaging of photoreceptors in 2 STGD1 patients[10].

Strengths of our study include that the data were from a large prospective multi-center international cohort of patients with molecularly confirmed ABCA4 associated STGD1. All testing and data collection was performed under standardized protocols. ProgStar had an independent data coordinating center which assured the data entry and management quality.

There are important limitations of our studies. First, SMART and ProgStar results do not generalize to STGD1 in general because of the ProgStar enrollment criteria. Only eyes with at least 1 well-demarcated area of atrophy with a minimum diameter of 300 μm and the total area of all atrophic lesions ≤ 12 mm^2 as determined by the clinical examiner were eligible. Thus, the ProgStar sample may have excluded both early stage and late stage STGD1 patients. Additionally, although the rates of mesopic sensitivity loss in the SMART study sample were comparable to the rate of mesopic sensitivity loss during 12-months reported in the ProgStar study sample of 359 eyes from 200 participants [40], the SMART study sample was a subsample of ProgStar, which may further reduces the generalizability of study findings. Nevertheless, generalizing to the complete ProgStar sample is less meaningful, rather, it is more important to interpret any ProgStar and SMART study finding in consideration of the characteristics of the patients sample specifically used in that analysis. Second, two-color perimetry tests

were not adopted in ProgStar or SMART, thus the exact loss of cone or rod function could not be determined. Third, at the time of our studies, all the Nidek MP-1S devices were running version number 1.7.7 of the NAVIS software, which required manual placement of the test pattern. This led to non-trivial proportions of tests deemed as poor pattern placement by the reading center and their data were not used in the analyses. Recent improved versions of the software allow automatic placement of the test pattern to match the foveal center as marked in an OCT B-scan passing through the foveal center, which should allow better centering of MP and most data being usable. Fourth, the longitudinal analysis was limited by the length of follow-up time (≤2 years) which may be insufficient to statistically differentiate the trajectories of scotopic and mesopic function losses, especially most eyes only contributed data over 12 or 18-months of follow-up. Normal aging is associated with faster loss of scotopic versus photopic sensitivity and faster loss of rod- versus cone-photoreceptor phototransduction[41,42]. But the differences are small especially during 1 to 2 years, and thus aging is unlikely to explain the faster loss of scotopic macular function in STGD1 observed here. Fifth, there were limits of detection using the Nidek MP1-S device. The MDs calculated here were the deviations from the maximally measurable normal using the MP-1S device. The mesopic MDs had a range of 0 to 20 dB. Such mMDs may be underestimating the deviations from normals due to the limit of detection for normals from the MP-1S device. The scotopic test used 1ND and 2ND filters which allowed a dynamic range of 0 to 40 dB in sMD. Following the study protocol, most of the scotopic tests used 1ND filter to accommodate the impaired visual function in STGD1 patients. However, for test loci that could afford the normal response of 20dB under the 1ND filter, their values of deviations

were calculated as 10dB because of the limit of detection under the 1ND filter. Of the 386 scotopic tests that used 1ND filter, a quarter of the tests had more than 20% of the test loci that had a normal response of 20dB. Therefore, it was possible that the sMD may be overestimated for eyes that had better scotopic macular function, resulting an over estimation of the difference between scotopic and mesopic function losses. Nevertheless, we conducted a sensitivity analysis to correct for the overestimation by imputing the deviation values to be 0dB for the test loci that had a normal response of 20dB under 1ND filter, and the study conclusions remain unchanged (Supplemental Figure 1 shows the comparison of imputed extrafoveal sMDs to mMDs).

In conclusion, our data suggest that in STGD1, in the extrafovea, scotopic macular function loss preceded mesopic function loss and proceeded at a faster speed. Using sMD as a primary outcome measure may help enroll earlier stage patients and sMD provides a direct functional outcome for testing the efficacy of therapeutics that target on preserving rod photoreceptors. Scotopic and mesopic macular sensitivity measures provide alternative visual function outcomes that are more sensitive to detect change than BCVA for designing future treatment trials aimed to slow progression in STGD1.

Table of Contents Statement

This multicenter study followed 127 patients with molecularly confirmed *ABCA-4* gene mutation associated Stargardt disease for up to 2 years. The study found that scotopic macular function loss was earlier and faster than mesopic macular function loss. Scotopic and mesopic macular sensitivities using microperimetry provide viable visual function outcomes for treatment trials of *ABCA-4* gene associated Stargardt disease.

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b. Financial Disclosures

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Isabelle Audo: Consultancy with Novartis, SparingVision, Biogen

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Michael Ip: Consultant for: Novartis, Genentech, Allergan, Regeneron, RegenexBio, Apellis, Aerie, Alimera, Amgen, Cell Lineage Therapeutics, clearside, OCCURX

SriniVas Sadda: Consultancy: Allergan, Amgen, Genentech/Roche, Novartis, 4DMT, NGM Bio, Regeneron, Oxurion, Heidelberg, Merck, Centervue, Optos. Research Instruments: Nidek, Topcon, Optos, Heidelberg Engineering, CenterVue, Carl Zeiss Meditec.

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Hendrik Scholl is member of the Scientific Advisory Board of: Astellas Institute for Regenerative Medicine; Gensight Biologics; Ionis Pharmaceuticals, Inc.; Gyroscope Therapeutics Ltd.; Janssen Research & Development, LLC (Johnson & Johnson); and Pharma Research & Early Development (pRED) of F. Hoffmann-La Roche Ltd; Novartis Pharma AG (CORE). Dr. Scholl is paid consultant of: Boehringer Ingelheim Pharma GmbH & Co; Gerson Lehrman Group; and Guidepoint.

Hendrik Scholl is member of the Data Monitoring and Safety Board/Committee of Belite Bio and ReNeuron Group Plc/Ora Inc. and member of the Steering Committee of Novo Nordisk (FOCUS trial).

Hendrik Scholl is co-director of the Institute of Molecular and Clinical Ophthalmology Basel (IOB) which is constituted as a non-profit foundation and receives funding from the University of Basel, the University Hospital Basel, Novartis, and the government of Basel-Stadt.

These arrangements have been reviewed and approved by the University of Basel (Universitätsspital Basel, USB) in accordance with its conflict of interest policies.

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Appendix: The SMART Study Group Members

The SMART studies consist of the Chair's Office, Data Coordinating Center, Image Reading Center, and six clinics with the following members:

Chair's Office
Hendrik P.N. Scholl, MD; MA
Rupert W. Strauss, MD
Yulia Wolfson, MD
Millena Bittencourt, MD
Syed Mahmood Shah, MD
Mohamed Ahmed, MD
Etienne Schönbach, MD
Kaoru Fujinami, MD, PhD

Dana Center Data Coordinating Center
Sheila K. West, PhD
Ann-Margret Ervin, PhD
Beatriz Munoz, MS
Xiangrong Kong, PhD
Kurt Dreger, BS
Jennifer Jones, BA
Robert Burke, BA

Greater Baltimore Medical Center, Towson
Janet S. Sunness, MD
Carol Applegate, MLA, COT
Leslie Russell, Mac
Jasmine Foster

Moorfields Eye Hospital, London
Michel Michaelides, MD
Simona Degli Esposti, MD
Anthony Moore
Andrew Webster, MD
Sophie Connor, BSc
Rupert W Strauss, MD
Jade Barnfield, BA
Zaid Salchi, MD
Clara Vasquez-Alfageme, MD
Victoria McCudden
Maria Pefkianaki, MD
Jonathan Aboshiha, MA, MB
Gerald Liew
Graham Holder, PhD

Anthony Robson, PhD
Alexa King, BA
Daniela Ivanova Cajas Narvaez, MSc
Katy Barnard, BS
Catherine Grigg, BSc
Hannah Dunbar, PhD
Yetunde Obadeyi
Karine Girard-Claudon, MST
Hilary Swann, BSc
Avani Rughani, BSc
Charles Amoah, NVQ
Dominic Carrington
Kanom Bibi, BSc
Emerson Ting Co, DMD
Mohamed Nafaz Illiyas
Hamida Begum, BSc
Fiona Ellison, BSc, MSc
Sarah Hamburg, BSc
Selma Lewis, BSc
Racehl Murphy, BSc
Lauren Perkins, BSc
Amar Singh, BSc
Kaoru Fujinami, MD
Anthonay Robson, PhD
Andrew Carter, BSc
Anne Georgiou, PhD
Selma Lewism BSc
Saddaf Shaheen, PGDip, BSc
Harpreet Shinmar, MSc
Linda Burton, BSc

Retina Foundation of the Southwest, Dallas
David Birch, PhD
Yi-Zhong Wang, PhD
Luis Rodriguez, BS
Kirsten Locke, BS
Martin Klein, MS
Paulina Mejia, BS

University of Tübingen, Tübingen
Eberhart Zrenner, PhD
Fadi Nasser, MD
Gesa Astrid Hahn, MD
Barbara Wilhelm, MD

Tobias Peters, MD
Benjamin Beier, BSc
Lars Genedy
Matthias Deitermann
Regine Grund
Susanne Kramer, Dipl. Biol.

The Vision Institute, Paris
José-Alain Sahel, MD
Saddek Mohand-Said, MD, PhD
Isabelle Audo, MD, PhD
Caroline Laurent-Coriat, MD
Ieva Sliesoraityte, MD, PhD
Christina Zeitz, PhD
Fiona Boyard, BS
Minh Ha Tran, BS
Mathias Chapon, COT
Céline Chaumette, COT
Juliette Amaudruz, COT
Victoria Ganem, COT
Serge Sancho, COT
Aurore Girmens, COT

The Wilmer Eye Institute, Baltimore
Hendrik P.N. Scholl, MD
Rupert W. Strauss, MD
Mandeep Singh, MD
Yulia Wolfson, MD
Syed Mahmood Shah, MD
Mohamed Ahmed, MD
Etienne Schönbach, MD
Robert Wojciechowski, PhD
Shazia Khan, MD
David G. Emmert, BA
Dennis Cain, CRA
Mark Herring, CRA
Jennifer Bassinger, COA
Lisa Liberto, COA

Doheny Image Reading Center
Srinivas Sadda, MD
Michael S. Ip, MD
Anamika Jha, MBS
Alex Ho, BS

Brendan Kramer, BA
Ngoc Lam, BA
Rita Tawdros, BS
Yong Dong Zhou, MD, PhD
Johana Carmona, HS
Akihito Uji, MD, PhD
Amirhossein Hariri, MD
Amy Lock, BS
Anthony Elshafei, BS
Anushika Ganegoda, BS
Christine Petrossian, BS
Dennis Jenkins, MPH
Edward Strnad, BS
Elmira Baghdasaryan, MD
Eric Ito, OD
Feliz Samson, BS
Gloria Blanquel, BS
Handan Akil, MD, FEBOpht
Jhanisus Melendez, MS
Jianqin Lei, MD
Jianyan Huang, MD, PhD
Jonathan Chau, BS
Khalil G. Falavarjani, MD
Kristina Espino, BS
Manfred Li, BS
Maria Mendoza, BS
Muneeswar Gupta Nittala, MPhil Opt
Netali Roded, BS
Nizar Saleh, MD
Ping Huang, MD, PhD
Sean Pitetta, BS
Siva Balasubramanian, MD, PhD
Sophie Leahy, BA
Sowmya J. Srinivas, MBBS
Swetha B. Velaga, B Opt
Teresa Margaryan, BA
Tudor Tepelus, PhD
Tyler Brown, BS
Wenying Fan, MD
Yamileth Murillo, BA
Yue Shi, MD, PhD
Katherine Aguilar, BS
Cynthia Chan, BS
Lisa Santos, HS

Brian Seo, BA
Christopher Sison, BS
Silvia Perez, BS
Stephanie Chao, HS
Kelly Miyasato, MPH
Julia Higgins, MS
Zoila Luna , MHA
Anita Menchaca, BS
Norma Gonzalez, MA
Vicky Robledo, BS
Karen Carig, BS
Kirstie Baker, HS
David Ellenbogen, BS
Daniel Bluemel, AA
Theo Sanford, BS
Daisy Linares, HS
Mei Tran, BA
Lorane Nava, HS
Michelle Oberoi, BS
Mark Romero, HS
Vivian Chiguil, HS
Grantley Bynum-Bain, BA
Monica Kim, BS
Carolina Mendiguren, MEM
Xiwen Huang, MPH
Monika Smith, HS

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Figure captions

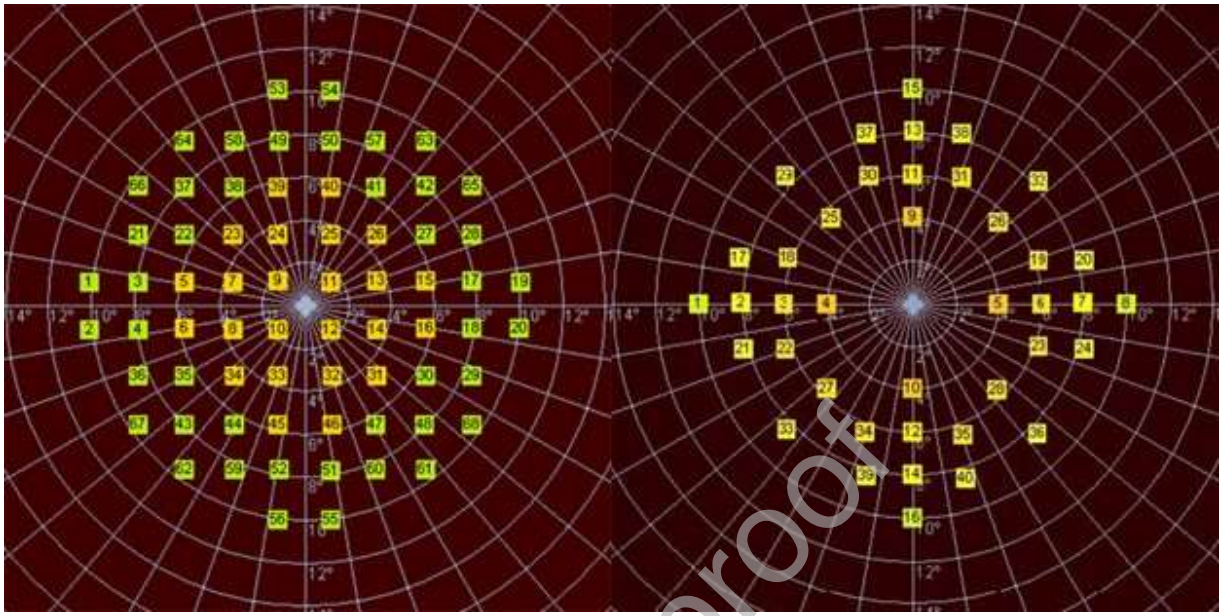


Figure 1. Distributions of microperimetry (MP) test loci in ProgStar and SMART studies. Left: Mesopic MP test pattern including 68 loci from 2° to 10°. Right: Scotopic MP test pattern including 40 loci from 4° to 10°.

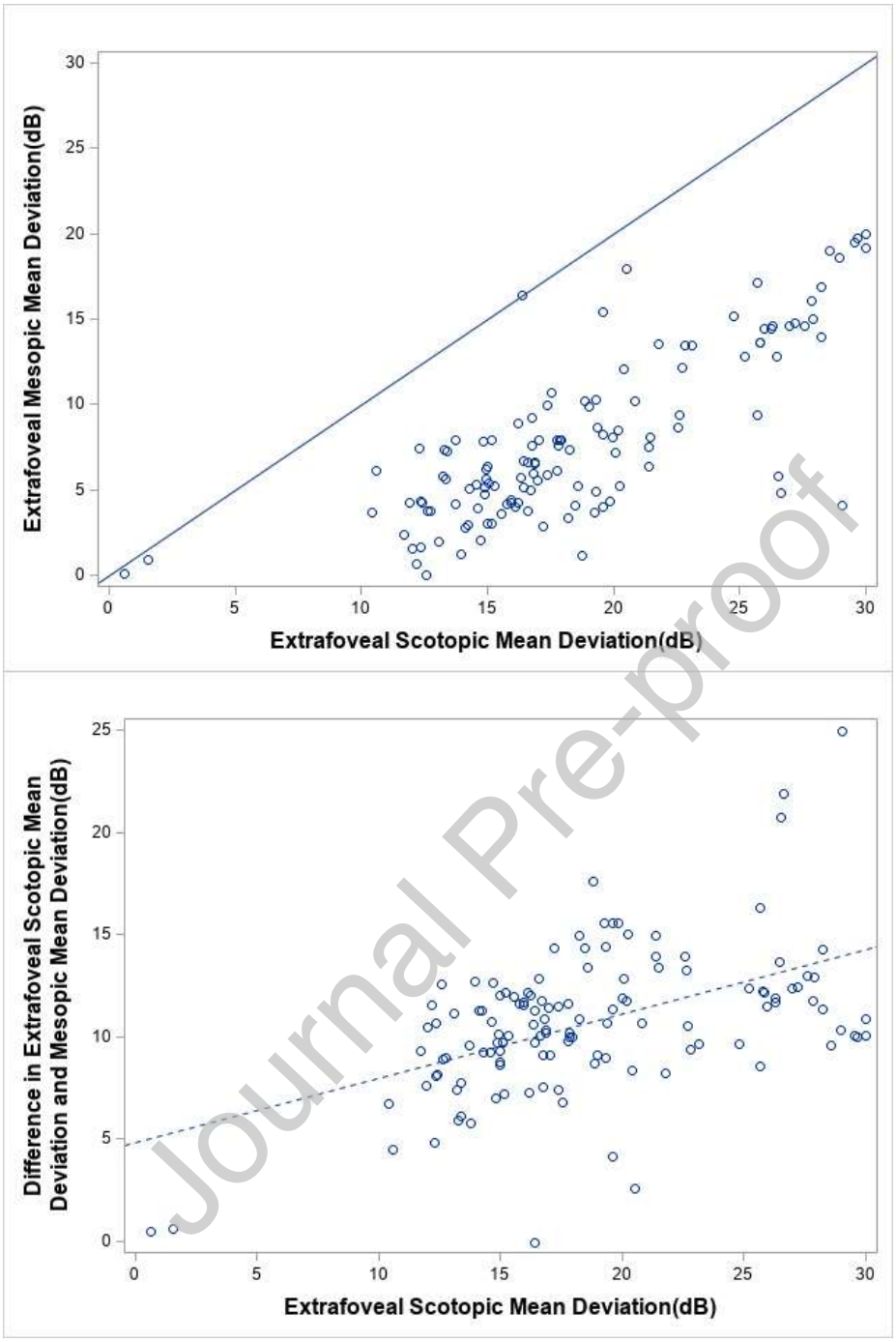
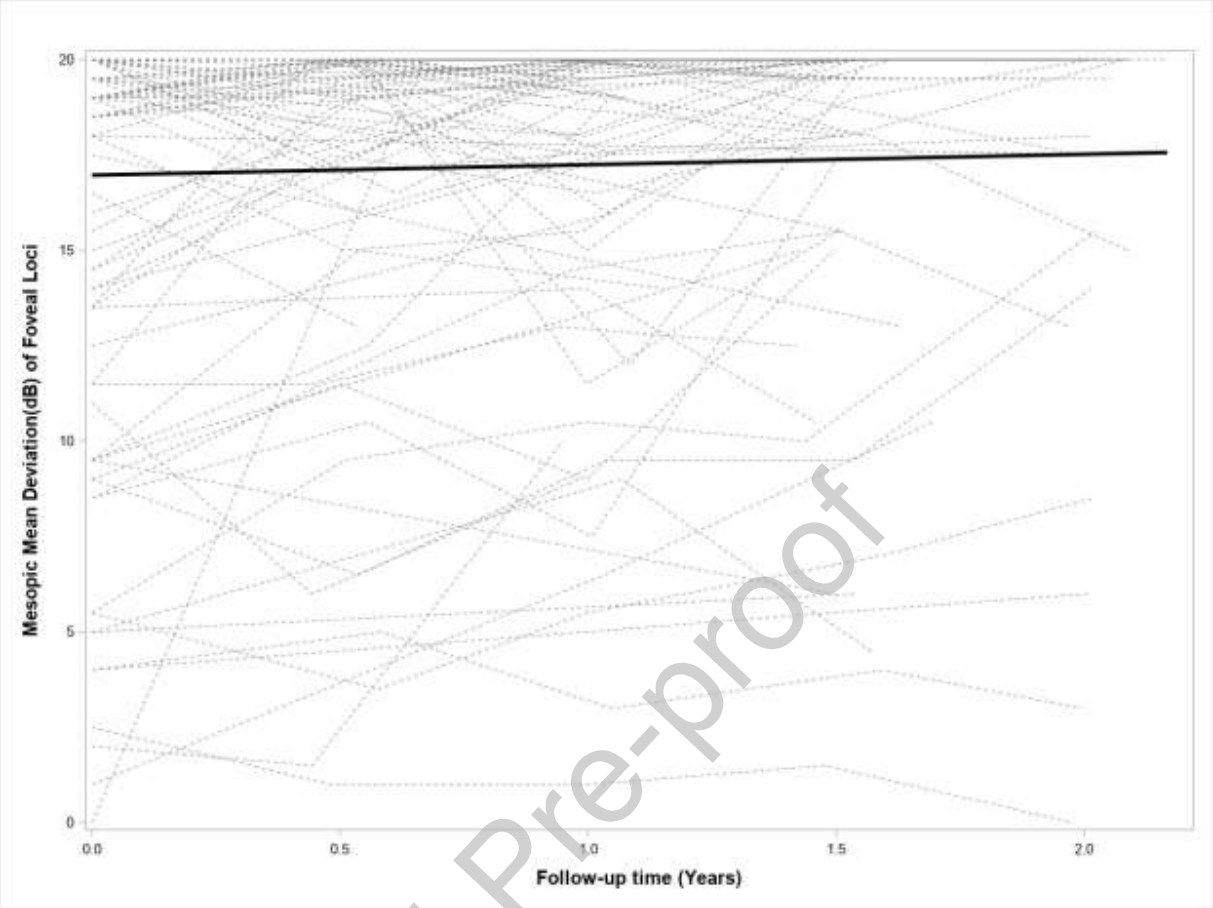


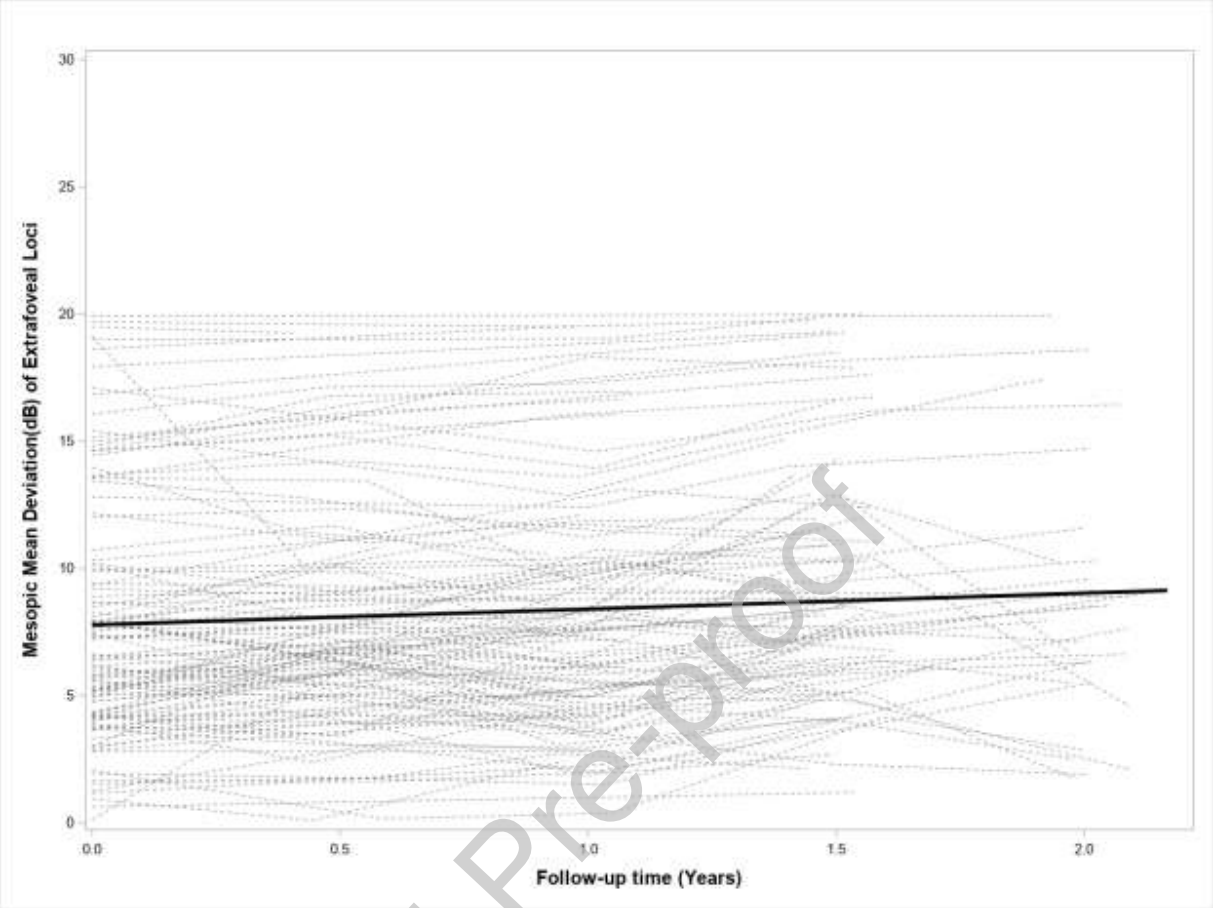
Figure 2. Plots of cross-sectional data at the SMART baseline visit.

A: Scatter plot of the cross-sectional extrafoveal scotopic mean deviation (MD) versus extrafoveal mesopic MD at SMART baseline visit. The line is the diagonal indicating equal x-axis and y-axis values.

B: Scatter plot of the difference between extrafoveal mMD and sMD versus sMD at the baseline visit. The dotted line is the fitted line from the linear regression 0.32 (95%CI: 0.20 to 0.43, p<.001). The positive linear trend means that the larger the

scotopic MD, the greater difference between extrafoveal mesopic and scotopic responses and loss of scotopic response was always greater than loss of mesopic response.





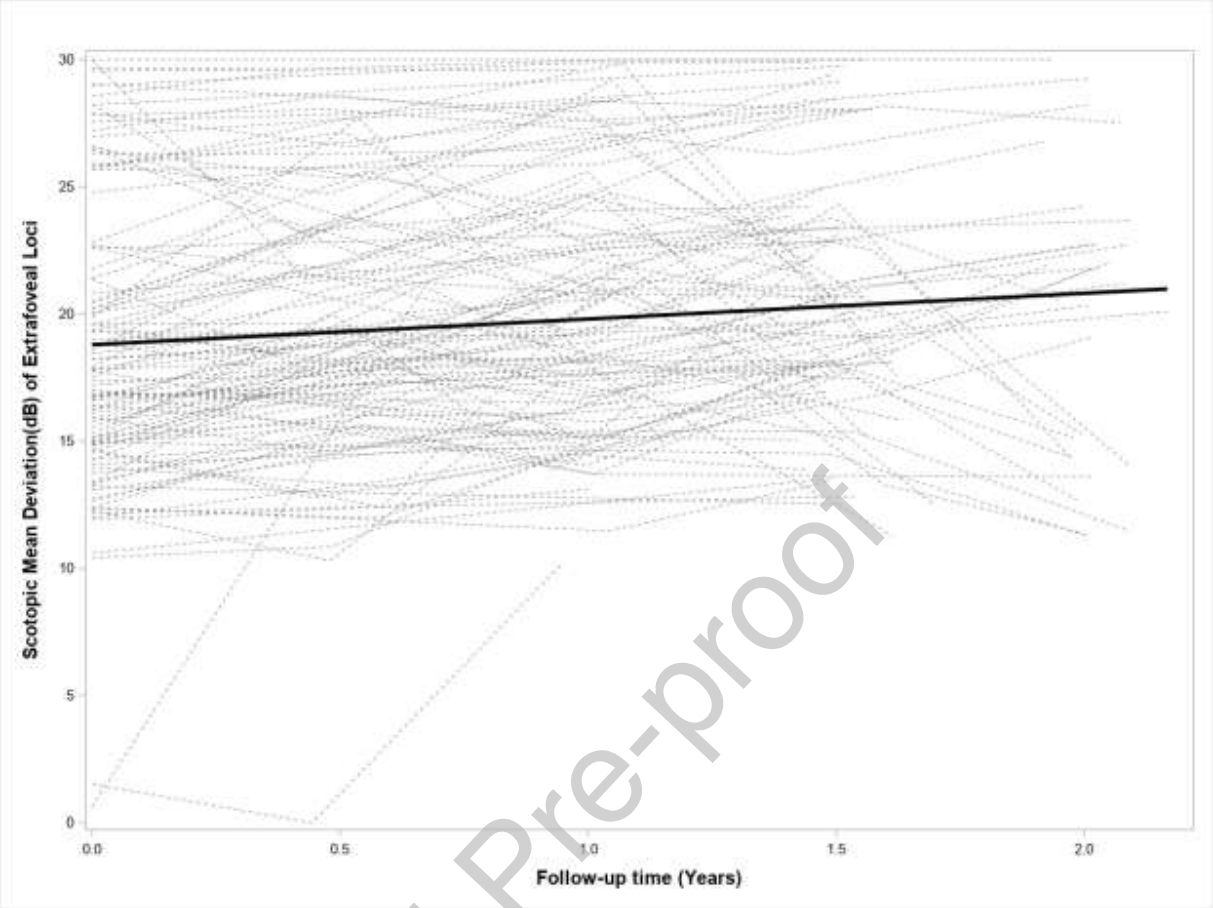


Figure 3. Spaghetti plots showing the longitudinal trajectories of mean deviations (MD) during the follow-up. Each dotted line shows observed data for one eye. The real line is the trend of average MD over time estimated using linear mixed effects model.

A. Mesopic MD of the fovea loci. The slope estimate is 0.72 (95%CI: 0.37 to 1.07) dB/year.

B. Mesopic MD of the extrafoveal loci. The slope estimate is 0.86 (95%CI: 0.58 to 1.14) dB/year.

C. Scotopic MD of the extrafoveal loci. The slope estimate is 1.12 (95%CI: 0.66 to 1.57) dB/year.

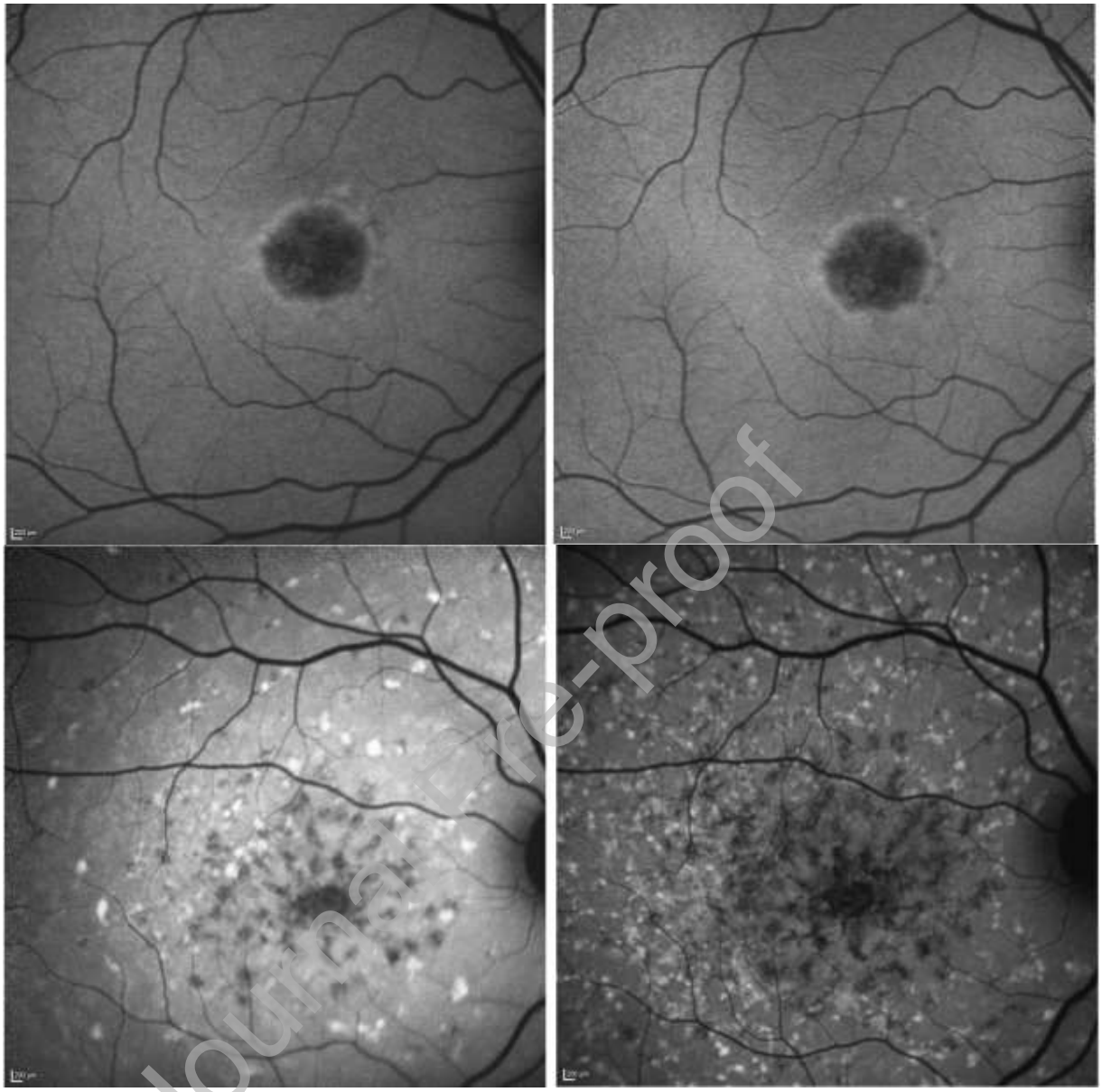


Figure 4. Fundus autofluorescence (FAF) images of the 2 eyes that had normal extrafoveal mesopic and scotopic sensitivity responses at SMART baseline.

A. FAF image of Eye 1 in Table 3 acquired at the SMART baseline visit (left) and the image acquired approximate 12-months after the SMART baseline visit (right).
B. FAF image of Eye 2 in Table 3 acquired at the SMART baseline visit (left) and image acquired at approximate 18-months after the SMART baseline visit (right).

Table 1. Baseline demographics and clinical characteristics of participants enrolled in the SMART study.

		Number *(n=127)*	%		
Gender					
Female		66	52.0		
Race					
White/Caucasian		105	82.7		
Black/African American		12	9.5		
Asian		7	5.5		
Unknown		3	1.6		
Age at onset					
≤ 18		53	41.7		
>18		68	53.5		
unknown		6	4.7		
	Mean	**SD**	**Median**	**IQR**	**Range**
Age at Baseline (years)	34.5	15.1	33.0	22.0-45.0	11.0-70.0
Duration of symptoms (years)	10.1	7.0	8.5	5.5-13.5	0-30.0
Age at onset among known (years)	24.7	14.5	21.0	13.0-36.0	4.0-64.0
Best corrected visual acuity (ETDRS Letters) (Snellen equivalent)	52.0 (20/91)	18.6	45.0 (20/126)	39.0-70.0 (20/166-20/40)	19.0-93.0 (20/417 to 20/14)
Mean Deviations (MD) (dB) in all eyes (N=127)					
Overal Mesopic MD (central 20°)	8.36	4.79	7.20	4.80 to 10.80	0 to 19.90
Mesopic MD in the fovea (loci of 2° eccentricity)	16.78	5.32	19.50	15.00 to 20.00	0 to 20.00
Mesopic MD Extrafovea (loci of 4-10° eccentricity)	7.91	4.90	6.59	4.22 to 10.31	0 to 19.94
Scotopic MD Extrafovea (loci of 4-10° eccentricity)	18.59	5.60	17.40	14.90 to 21.78	0.63 to 30.00
Extrafovea Difference*: Scotopic MD-Mesopic MD	10.68	3.53	10.69	9.09 to 12.21	-0.03 to 24.93
Mean Deviations (MD) in eyes with approximately normal extrafovea mMD at baseline (N=81)					
Mesopic MD in the fovea	15.74	6.00	19.0	11.5 to 20.0	0 to 20

Scotopic MD Extrafovea	15.72	4.07	15.55	13.73 to 17.75	0.63 to 29.05
Mean Deviations (MD) in eyes with approximately normal fovea mMD at baseline (N=11)					
Mesopic MD in the extrafovea	2.67	2.89	1.66	0.63 to 3.69	0-9.34
Scotopic MD Extrafovea	12.67	7.06	12.58	10.43 to 15.56	0.63 to 25.68

IQR= interquartile range, SD = standard deviation. MD=Mean deviation from maximally measurable normal under Nidek MP-1S

*: Positive difference between scotopic and mesopic MDs means larger impairment in sMD.

Table 2. Rates of declines in foveal mesopic and extrafoveal mesopic and scotopic mean deviations. mMD: mesopic mean deviation. sMD: scotopic mean deviation. CI: confidence interval.

	Rates		
	Foveal mMD (dB/year) (95%CI)	**Extrafovea mMD (dB) (95%CI)**	**Extrafovea sMD (dB) (95%CI)**
Among all eyes (N=127)	0.72 (0.37 to 1.07)	0.86 (0.58 to 1.14)	1.12 (0.66 to 1.57)
Among the eyes with approximately normal extrafovea mMD at baseline (N=81)	0.96 (0.47 to 1.46)	0.96 (0.61 to 1.32)	1.31 (0.74 to 1.89)
Among the eyes with approximately normal fovea mMD at baseline (N=11)	2.98 (-0.21, 6.18)	1.20 (-0.06 to 2.46)	1.58 (-0.84 to 4.00)

Table 3. Detailed data for the 2 eyes that had both scotopic and mesopic extrafoveal sensitivity above average normal at the baseline.

		Foveal mMD (dB)	**Extrafovea mMD (dB)**	**Extrafovea sMD (dB)**	**Atrophic lesion size in FAF (mm^2)***	**BCVA(ETDRS Letters)** (Snellen equivalent)
Eye 1	First visit	2.0	0.94	1.53	2.19	48 (20/110)
	Last visit (12-months follow-up)	10	2.41	10.20	2.25	49 (20/100)
Eye 2	First visit	0	0.13	0.63	0.57	45 (20/126)
	Last visit (18-months follow-up)	20.0	4.41	11.28	1.59	43 (20/138)

mMD: mesopic mean deviation.
sMD: scotopic mean diviation.
*: Atrophic lesion size was measured as the area of decreased autofluoresence
FAF: fundus autofluorescence
BCVA: best corrected visual acuity
ETDRS: Early Treatment Diabetic Retinopathy Study