Macular Function in Macular Degenerations: Repeatability of Microperimetry as a Potential Outcome Measure for *ABCA4*-Associated Retinopathy Trials

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PURPOSE. To measure macular visual function in patients with unstable fixation, to define the photoreceptor source of this function, and to estimate its test-retest repeatability as a prerequisite to clinical trials.

METHODS. Patients ($n = 38$) with *ABCA4*-associated retinal degeneration (RD) or with retinitis pigmentosa (RP) were studied with retina-tracking microperimetry along the foveo-papillary profile between the fovea and the optic nerve head, and point-by-point test-retest repeatability was estimated. A subset with foveal fixation was also studied with dark-adapted projection perimetry using monochromatic blue and red stimuli along the horizontal meridian.

RESULTS. Macular function in *ABCA4*-RD patients transitioned from lower sensitivity at the parafovea to higher sensitivity in the perifovea. RP patients had the inverse pattern. Red-on-red microperimetric sensitivities successfully avoided ceiling effects and were highly correlated with absolute sensitivities. Point-by-point test-retest limits (95% confidence intervals) were ± 4.2 dB; repeatability was not related to mean sensitivity, eccentricity from the fovea, age, fixation location, or instability. Repeatability was also not related to the local slope of sensitivity and was unchanged in the parapapillary retina.

CONCLUSIONS. Microperimetry allows reliable testing of macular function in RD patients without foveal fixation in longitudinal studies evaluating natural disease progression or efficacy of therapeutic trials. A single estimate of test-retest repeatability can be used to determine significant changes in visual function at individual retinal loci within diseased regions that are homogeneous and those that are heterogeneous and also in transition zones at high risk for disease progression. (*Invest Ophthalmol Vis Sci.* 2012;53:841–852) DOI:10.1167/iovs.11-8415

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Supported by National Eye Institute Grant EY 013203 (AVC), Foundation Fighting Blindness, Hope for Vision, and Macula Vision Research Foundation. AVC is an RPB Senior Scientific Scholar.

Submitted for publication August 14, 2011; revised November 27, 2011; accepted January 1, 2012.

Disclosure: A.V. Cideciyan, None; M. Swider, None; T.S. Aleman, None; W.J. Feuer, None; S.B. Schwartz, None; R.C. Russell, None; J.D. Steinberg, None; E.M. Stone, None; S.G. Jacobson, None

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Mutations in the *ABCA4* gene form one of the most common molecularly identified causes of autosomal recessive retinal degenerations (RDs), often categorized clinically as Stargardt disease or cone-rod dystrophy.^{1–7} The disease expression of *ABCA4*-RD can cover a wide spectrum from maculopathies involving only the foveal/parafoveal region to severe retina-wide disease.^{8–12} Importantly, all stages and severities of *ABCA4*-RD published to date demonstrate retinal degeneration involving the macula, unlike the typical form retinitis pigmentosa (RP), in which central macular structure and function can be normal or mildly affected until later stages of disease.¹³ The consequences of visual dysfunction resulting from macular degeneration are extremely important to patients' daily living. Thus, treatment strategies for *ABCA4*-RD currently in development using pharmaceutical, stem cell, and gene therapy approaches will require quantitative and reliable outcome measures of macular function to define the possible amelioration of the progression of the disease. One choice of outcome involves psychophysical measurements of stimulus detection thresholds at defined retinal loci.

Conventional computerized projection-type threshold perimetry is performed under free viewing conditions in which the subject is asked to look at a fixation point and the correspondence between stimuli presented within the subject's field of view and the retinal coordinates are estimated from assumptions on the retinal location of fixation and the angular magnification of the eye. This approach works well when fixation is stable and foveal, such as in *ABCA4*-RD with foveal preservation.¹¹ However, most *ABCA4*-RD involves macular degeneration that includes foveal degeneration, lost stable fixation, and significant challenges to measuring macular function with conventional perimetry. Retina-tracking microperimetry, on the other hand, is independent of the location and stability of fixation because it uses images of the retina obtained in real time to place stimuli repetitively on the same set of retinal locations within a prespecified spatial pattern to obtain a psychophysical threshold.^{14,15} This technique has been used in different forms of macular disease,^{14,16–27} including some patients with *ABCA4*-RD.^{10,28,29} Here we first designed a microperimetric pattern that balances considerations of testing time and spatial resolution. This pattern was found to be appropriate for a wide range of disease stages encountered in *ABCA4*-RD. Then we evaluated the relationship between microperimetric thresholds and conventional thresholds. Last, we estimated the test-retest repeatability of this microperimetric test of macular function. Our repeatability estimates provide data that define the significance of changes in visual function with disease progression at a given retinal locus. The stage is thus set for determining the safety and efficacy of treatments

TABLE 1. Clinical and Molecular Characteristics of the *ABCA4* Patients

Patient	Age (y)/Sex	ABCA4 Mutation		Clinical Diagnosis	Visual Acuity*	Kinetic Visual Field Extent (V-4e)†
		Allele 1	Allele 2			
Foveal Fixation						
P1‡	12/M	N965S	W821R	STGD	20/20	97
P2‡	17/F	V989A	IVS28+5 G>T	STGD	20/100	90
P3	18/M	G1961E	R1129L§	STGD	20/100	105
P4	21/F	R212C	P68R	STGD	20/125	101
P5	24/M	P1511 del1ccgC	R1705Q	STGD	20/25	114
P6	31/M	G863A	R1108C	STGD	20/25	105
P7	32/F	IVS40+5 G>A	V935A	STGD	20/32	103
P8	34/M	G1961E	—	CRD	20/32	98
P9	37/F	R681X	P309R	STGD	20/20	109
P10	39/M	G1961E	C54Y§	STGD	20/40	101
P11‡	42/F	G1961E	V256V	STGD	20/32	105
P12‡	46/F	G1961E	V256V	STGD	20/32	106
P13	52/F	G1961E	P1380L	STGD	20/40	105
P14	58/M	D600E	R18W§	STGD	20/40	84
Extrafoveal Fixation						
P15	11/M	V256V	T1526M	CRD	20/200	102
P16	15/M	C54Y	IVS35+2 T>C	STGD	20/200	96
P17‡	16/F	V989A	IVS28+5 G>T	STGD	20/100	100
P18‡	16/M	N965S	W821R	STGD	20/125	100
P19	19/F	A1038V/L541P	N965S	STGD	20/400	90
P20	21/M	G863A	IVS35+2 T>C	STGD	20/200	99
P21	22/F	G1961E	R152X	STGD	20/50	104
P22	27/M	G863A	P1660S§	STGD	20/100	98
P23	27/F	G1961E	A1038V/L541P	STGD	20/100	109
P24	29/M	G1961E	T1019M	STGD	20/100	104
P25	33/M	P1486L	deletion of exon 7	STGD	20/400	98
P26	36/F	G863A	C1490Y	STGD	20/100	93
P27	41/M	A1038V/L541P	—	STGD	20/125	108
P28	49/F	T1526M	R2030Q	STGD	20/125	98
P29	55/F	W855X	—	STGD	20/160	87
P30	56/F	G1961E	IVS37+1 G>A§	STGD	20/125	89
P31	60/F	G1961E	M669 del2ccAT	STGD	20/125	104

STGD, Stargardt disease; CRD, cone-rod dystrophy.

* Best-corrected visual acuity of the tested eye.

† Expressed as a percentage of normal mean of V-4e target; 2 SD below normal equals 90%.

‡ Indicates sibling pairs: P1 and P18, P2 and P17, P11, and P12.

§ Phase unknown.

that aim to improve vision in the short term or to slow or arrest the natural progression of the disease in the longer term.

SUBJECTS, MATERIALS, AND METHODS

Subjects

The study population consisted of 38 eyes of 38 patients in two groups; the larger group ($n = 31$, ages 11–60) had a clinical diagnosis within the spectrum of Stargardt disease or cone-rod dystrophy caused by *ABCA4* mutations (Table 1). A smaller group ($n = 7$, ages 17–67 years) had a diagnosis of RP with no known molecular cause to date (Table 2). A complete eye examination was performed in all subjects, including best-corrected ETDRS visual acuity and Goldmann kinetic perimetry. Normal data for microperimetry were collected from subjects with normal vision ($n = 8$; ages 21–59 years). Informed consent was obtained for all subjects; procedures followed the Declaration of Helsinki and were approved by the institutional review board.

Retina-Tracking Microperimetry and Fixation

A commercially available retina-tracking microperimeter (MP1; Nidek Inc., Fremont, CA) was used as described previously.^{10,30} Stimuli were red (maximum luminance, 127 cd/m²) and Goldmann III-sized (0.43° diameter), had a 200-ms duration, and were presented on a red back-

ground (1 cd/m²). Patients' eyes were dilated and fully dark-adapted. Testing was performed unilaterally in a dark room with a black curtain blocking any stray light originating from the computer screen on the examiner's side from reaching the subject's eye. A custom perimetric

TABLE 2. Clinical Characteristics of the RP Patients

Patient	Age (y)/Sex	Clinical Diagnosis*	Visual Acuity†	Kinetic Visual Field Extent (V-4e)‡
P1	17/F	ADRP	20/20	103
P2	20/F	RP, multiplex	20/40	24
P3	27/M	RP, simplex	20/20	30
P4	46/M	RP, simplex	20/32	6
P5	47/M	RP, simplex	20/20	93
P6	58/F	RP, simplex	20/20	4
P7	67/F	RP, simplex	20/25	9

RP, retinitis pigmentosa; ADRP, autosomal dominant retinitis pigmentosa.

* Patients were screened, and results were negative for relevant RP genes.

† Best-corrected visual acuity of the tested eye.

‡ Expressed as a percentage of normal mean of V-4e target; 2 SD below normal equals 90%.

test pattern (named foveo-papillary profile [FPP]) was designed.¹⁰ The advantages of FPP include the following: (a) high sampling density homogeneously covering para- and perifoveal regions and the parapapillary region, (b) view of the optic nerve head (ONH) region with available high-contrast targets to be tracked, (c) inclusion of the physiological blind spot (pBS) providing a known deep scotoma boundary independent of disease severity, (d) lack of major blood vessels along the horizontal meridian avoiding possible angioscotomas, and (d) existence of definite landmarks on OCT (such as fovea and ONH) that allow easier registration of OCT with en face imaging methods. FPP pattern consisted of 31 loci placed along the horizontal meridian starting 1° nasal to the fovea and extending nasally to 16° eccentricity (crossing the ONH) with 0.5° sampling intervals. The same examiner performed all tests in all subjects. In a pretest, preferred retinal location used for fixation was determined for each patient. If fixation was found to be extrafoveal, FPP was placed manually to correspond to the anatomic location of the fovea, taking advantage of the foveal or parafoveal atrophy visible on the near-infrared view. The “show tracking reference” option was turned on to visually confirm that the selected landmark was being tracked throughout the test. Threshold estimates were fully automated using the 4-2 staircase strategy, as suggested by the manufacturer.³¹ For the initial test, thresholds were set to start at 5 dB. For repeat testing, the “follow-up” function built in to the MP1 software was used. Raw threshold data were exported, and a 3-point spatial moving average was applied to compensate for the spatial resolution of the MP1 system. Raw fixation data and the near-infrared (NIR) image acquired by MP1 were exported to calculate the statistics for estimating the eccentricity and instability of fixation.

Dark-Adapted Static Perimetry

In the subset of patients with foveal fixation, visual sensitivities under dark-adapted conditions were determined with a modified computerized projection perimeter (Humphrey Field Analyzer; Zeiss Meditec, Dublin, CA) as previously published.³² The stimuli were monochromatic blue (500 nm) or red (650 nm), Goldmann V-sized (1.7° in diameter) with 200-ms duration. The horizontal meridian crossing fixation was sampled at 2° intervals to an eccentricity of 30°. Photoreceptor mediation was defined using sensitivity differences to the two stimuli, and sensitivity losses were defined compared with normal results under dark-adapted or cone-plateau conditions.

En Face and Cross-Sectional Imaging

Spatial topography of retinal pigment epithelium (RPE) health was estimated with a reduced-illumination autofluorescence imaging (RAFI) method, which minimizes the absorption of imaging light by rod and cone opsins and lipofuscin and thus the likelihood of accelerating the natural history of the disease.¹⁰ Specifically, a confocal scanning laser ophthalmoscope (HRA2 or Spectralis HRA without OCT; Heidelberg Engineering GmbH, Heidelberg, Germany) was used to image with a 790-nm NIR excitation light and a long-pass filter for >805 nm. The resultant NIR-RAFI signal is believed to be dominated by the melanolipofuscin in RPE and melanin in the RPE and choroid.^{10,33} NIR-RAFI is thought to be more sensitive to early stages of *ABCA4* disease than the traditional short-wavelength autofluorescence.^{10,34,35} Retinal cross-sections were made with a spectral-domain (SD) OCT system (RTVue-100; Optovue Inc., Fremont, CA), as previously published.^{36–38}

Statistical Analysis

Signed (second test minus first test) and absolute test-retest differences were examined for trends in magnitude and variability with Bland-Altman plots.³⁹ A variance component analysis incorporating patients and eccentricity nested within patients and test-retest as random effects was performed to estimate test-retest SD (TRTSD) accounting for multiple measurements made on the same patient. The results were used to calculate pointwise 95% test-retest limits ($\pm 1.96 \times \sqrt{2} \times \text{TRTSD}$), as previously published.⁴⁰ The relationship of TRTSD to the following parameters was assessed: fixation, age, local slope, and

eccentricity from the physiological blind spot. Separate test-retest variances were calculated for foveal and extrafoveal fixating groups, and these were compared with an F-test. Because age and fixation were patient-level variables, variance component analyses were performed for each eye to generate individual TRTSD estimates. Relationships between each of these variables were evaluated with scatterplots and Pearson correlations. Mixed linear models with the patient included as a random effect were used to evaluate the dependence of TRTSD on local slope and eccentricity from the blind spot. An additional variance components analysis was performed on the RP patients, and their TRTSD was compared to that of the *ABCA4*-RD patients with an F-test.

MP1 sensitivities have a dynamic range limited to 20 dB (2 log₁₀ units), which makes ceiling and floor effects an important consideration when estimating repeatability.²⁴ Ceiling effects (subject seeing the dimmest, 20-dB, stimulus) are commonly observed under standard white-on-white testing conditions.^{16,21,24,25,29,41–43} In contrast, with our choice of red-on-red testing conditions,¹⁰ no ceiling effects were observed. Specifically, the highest sensitivity recorded was 18 dB. Floor effects (subject not seeing the brightest, 0-dB, stimulus) were often encountered in subjects with normal vision (because of the physiological blind spot) and in patients. In general, we excluded all data with floor effects from further analyses. An exception was retinal locations at which the MP1 stimulus was recorded as not seen for one test and a threshold was obtained on a second test. We observed that nearly all these locations were near a relative or an absolute scotoma boundary. We further observed that including these data *increased* the variability estimate instead of the hypothetical decrease in variability that would be expected because of floor effect. To be most conservative, we report overall repeatability results including these locations.

RESULTS

Macular Degeneration Spectrum in *ABCA4*-RD

ABCA4-RD can be associated with the full spectrum of macular health from the nearly normal retina to complete chorioretinal atrophy.^{1–12} Figure 1 illustrates some of the features of macular degeneration in *ABCA4*-RD using NIR-RAFI imaging to define RPE melanin abnormalities and OCT imaging to define retinal abnormalities in five patients. P6 and P24 (Table 1) exemplify some of the milder stages of macular disease involving the fovea (~5°-diameter circle centered on the foveola) or parafovea (~2°-wide annulus around the fovea) or both. There can be loss of RPE melanin associated with abnormal inner and outer segments; foveal regions can have partially retained photoreceptor nuclei (Fig. 1B) or complete atrophy (Fig. 1C). More advanced disease stages include greater involvement of photoreceptors and the RPE of the perifovea (~5°-wide annulus around the parafovea) as exemplified by P5 (Fig. 1D) or near complete loss as exemplified by P20 and P25 (Figs. 1E, 1F). The extramacular retina can appear to be normal (Figs. 1B, 1C), mildly involved (Fig. 1D), or severely affected (Figs. 1E, 1F). In severe stages of *ABCA4*-RD, an annular parapapillary region is often retained immediately outside the macula. Previous studies have shown the relative preservation of this region structurally and functionally.^{9,10,44,45} P5, P20, and P25 illustrate the relative retention of the RPE melanin and photoreceptor structure in the parapapillary region (Figs. 1D–F).

Macular Function Testing Appropriate for the *ABCA4* Disease Spectrum

What is the appropriate sampling grid to use with retina-tracking perimetry to evaluate macular function in *ABCA4*-RD? Considering the spectrum of macular disease severity (Fig. 1), the parafoveal region should be densely sampled to detect any abnormal function at early disease stages, when most of the retina is normal. At intermediate stages of the disease, the peri-

FIGURE 1. Spectrum of macular disease severity caused by mutations in the *ABCA4* gene. NIR-RAFI (upper) and OCT (lower) imaging results shown in a healthy subject (A) and in five patients (B–F). Red horizontal lines on NIR-RAFI images demarcate the location of the OCT shown. The retinal layers corresponding to the ONL, IS/OS junction, and the RPE are colorized with blue, yellow, and orange, respectively. All NIR-RAFI images are individually contrast stretched for visibility of features.

foveal region should be densely sampled to reliably measure the expected centrifugal expansion of degeneration over time. At later stages of the disease, the parapapillary region should be sampled to register any detectable visual function. Although the parapapillary region is not strictly within the traditional definition of macula, it often becomes the preferred retinal locus for *ABCA4*-RD patients and thus represents an important “central” component of their vision.⁹ Densely sampling all macular regions in two dimensions would be technically possible but practically onerous because of the many hours of testing time that would be required. Thus standard patterns sample the macula at 2° intervals.^{14,15,17,20,21,23,24,27,29} An alternative approach involves a higher sampling density along one or more carefully selected meridians. We have previously proposed^{9,10} using an FPP along the horizontal meridian extending from the fovea nasally and crossing the ONH as one such alternative for *ABCA4*-RD.

Sensitivity Values along the FPP in Patients with *ABCA4*-RD and RP

FPP sensitivities in a representative *ABCA4*-RD patient are shown in a pseudocolor scale registered to the NIR-RAFI results (Fig. 2A). The patient is not able to perceive the brightest

available stimuli within the central atrophic region as well as within her pBS. Between 6° and 10° eccentricity from the fovea, she has abnormally reduced but detectable vision (Fig. 2A). We have previously reported the existence of a dysfunctional penumbra in structurally normal-appearing regions surrounding scotomas in other patients with *ABCA4*-RD.¹⁰ Of note, at and near the parapapillary boundary, she has normal sensitivity (Fig. 2A).

To better understand the range of macular dysfunction measurable, FPPs were performed in three groups of patients. The first group was the subset of *ABCA4*-RD patients ($n = 14$, ages 12–58; Table 1) with retained foveal fixation (Fig. 2B). At the parafovea, all sensitivities were abnormally reduced or undetectable, whereas at the perifovea the sensitivities could be normal or near normal. There was a tendency for sensitivities to increase with eccentricity along the FPP toward the parapapillary region (Fig. 2B). The second group was a more commonly encountered subset of *ABCA4*-RD patients ($n = 17$, ages 11–60; Table 1) who fixated using an extrafoveal retinal region (Fig. 2C). Parafoveal vision tended to be more severely affected in this group of patients, but sensitivities also increased with eccentricity as the parapapillary region was approached. Across the group of all *ABCA4*-RD, the mean eccen-

FIGURE 2. Sampling of the macular vision along the FPP with retina-tracking microperimetry. (A) Sensitivity values along the FPP coded to a pseudocolor scale and overlaid onto the NIR-RAFI image of a representative *ABCA4*-RD patient, P23. Gray bars represent loci where the brightest stimulus was not seen. (B–D) Results of FPP sensitivities measured in *ABCA4*-RD patients with (B) and without (C) foveal fixation and patients with RP (D). Gray lines delimit (mean $\pm$ 2 SD) normal sensitivities. Note, normal limits end at the greatest eccentricity (10°) where all subjects had measurable data. Normal sensitivity limits closer to the papillary boundary are shown in Figure 5. P23 results shown in (A) are highlighted (red) in (C).

tricity of first measurable threshold with MP1 was $6.2^\circ (\pm 3.2^\circ)$, and the eccentricity of the central edge of the physiological blind spot was $13.7^\circ (\pm 1.5^\circ)$ providing a $7^\circ (\pm 3.4^\circ)$ wide region of measurable thresholds. The third group was composed of patients with RP ($n = 7$, ages 17–67; Table 2) and with foveal and stable fixation. As expected, many of the RP patients showed normal or near-normal sensitivities at the parafoveal regions with increasing abnormalities with eccentricity (Fig. 2D), thus forming a control group to evaluate statistics of parafoveal vision often lacking in *ABCA4*-RD patients.

Source of the Visual Function Measured by Retina-Tracking Microperimetry

We determined the relationship of visual function measured with retina-tracking microperimetry to absolute sensitivities measured in a subset of patients; this subset had foveal fixation and thus allowed comparison with conventional projection perimetry at the same retinal locations along the FPP. Structural and functional data from P12, a representa-

tive of the group of *ABCA4*-RD patients with foveal fixation, are shown in Figure 3A. OCT shows abnormally thinned foveal and parafoveal regions with no detectable inner or outer segments but with still detectable ONL remaining at the fovea. MP1 sensitivity loss was shown as 20 dB in the parafoveal region where the patient was not able to see the brightest stimulus. Absolute thresholds show a 25-dB sensitivity loss consistent with MP1 results. At 4° of eccentricity, there are detectable but abnormal inner and outer segments and a thin ONL. MP1 sensitivity loss is 4 to 7 dB, and this is matched by the absolute sensitivity loss of 5 dB. From 6° eccentricity nasally, a nearly normal retinal structure is matched by normal or nearly normal sensitivities with both methods. Comparison of the absolute sensitivities to 500- and 650-nm stimuli showed rod (R) mediation at all loci except at 2° , which was indeterminate (Fig. 3A). Rod mediation of absolute thresholds at 650 nm suggests that MP1 thresholds could also be mediated by rods.

A representative of the group of RP patients with foveal fixation is P5 (Fig. 3B). There is relative structural preserva-

FIGURE 3. Comparison of sensitivity losses estimated with retina-tracking microperimetry and free viewing perimetry at corresponding retinal locations in the subset of patients with foveal fixation. (**A**, **B**) Results from representative patients with *ABCA4*-RD (**A**) and RP (**B**) demonstrate the relationship between retinal structure (*upper*) and retinal function (*lower*). Sensitivity losses with retina-tracking microperimetric testing (*small symbols*) are overlaid on those with free-viewing perimetric testing (*large symbols*). Symbol sizes correspond to stimuli sizes. *Triangles* indicate that the patient did not see the brightest available microperimetry stimulus at that retinal location. Photoreceptor mediation (R, rod; M, mixed; C, cone) for the absolute thresholds to 650-nm stimuli (shown) are determined by comparison to thresholds to 500-nm stimuli (not shown) measured at the same location. (**C**) Relationship between two estimates of sensitivity loss at all locations of all patients. *Solid black line*: linear regression; *gray lines*: 95% prediction intervals.

tion in the foveal and parafoveal regions surrounded by increasing photoreceptor degeneration with greater eccentricity toward the ONH, contrasting the pattern typically seen in the maculopathy of *ABCA4*-RD. Up to 6° eccentric MP1 thresholds and absolute thresholds to 650 nm were within the normal range. From 7° eccentricity onward, there

were increasing losses of sensitivity, but estimates from MP1 thresholds and absolute thresholds were similar (Fig. 3B). Comparison of the absolute sensitivities showed mixed (M) mediation (650-nm stimulus detection by cones and 500-nm stimulus detection by rods), implying that MP1 thresholds to red stimuli would be expected to be mediated by cones.

Data from all available retinal loci from all patients with foveal fixation (45 loci from 9 patients) were used to summarize the relationship between the loss of absolute sensitivity at 650 nm as measured with dark-adapted projection perimetry and the loss of increment sensitivity to the red stimulus as measured by microperimetry (Fig. 3C). Because of the differences in the sizes of the stimuli, three neighboring MP1 thresholds corresponded to each location of the absolute thresholds. For the comparison, the smallest loss of microperimetric sensitivity among the three neighbors was used assuming, in locally heterogeneous regions, the healthiest patch of retina illuminated would dominate the sensitivity to the larger stimulus. There were no obvious clusters formed by the diagnostic category (*ABCA4*-RD vs. RP) or by the type of photoreceptors mediating the absolute thresholds. Overall there was a strong ($r^2 = 0.74$) linear correlation. The associated regression line had an intercept near zero (1.9 dB), and the slope was 0.83, suggesting a small underestimate by the microperimetric increment sensitivity loss compared with the absolute sensitivity loss (Fig. 3C). Ideally the relationship may be expected to have a unity slope. Small deviations from unity slope may be related to the limited sample size. Additionally or alternatively, the smaller stimulus size and the dim mesopic background used in the microperimetry system could have contributed to the small deviation from unity slope.

Test-Retest Repeatability of Retina-Tracking Microperimetry Thresholds

To evaluate variability of microperimetric testing with the FPP pattern, we obtained two sets of thresholds either on the same day ($n = 21$) or within a short interval (6.9 ± 2.8 months; $n = 8$). P7 is a representative of the subset ($n = 7$) of *ABCA4*-RD patients with foveal fixation (Fig. 4A). The NIR-RAFI image shows the central area of demelanization surrounding a small island of foveal preservation (Fig. 4A, upper; foveal island is not visible because of overlaid fixation locations). The mean fixation location (Fig. 4A, white dot) is at the fovea, and a circle of 0.9° radius encompasses 95% of fixation variation in this patient (Fig. 4A, white circle). The two sets of FPPs obtained show reasonable concordance (Fig. 4A, lower) with test-retest differences ranging up to 4.7 dB at individual locations. A representative of the subset ($n = 15$) of *ABCA4*-RD patients with extrafoveal fixation is P31 (Fig. 4B). The NIR-RAFI image shows a central region of RPE demelanization that has unmasked the choroidal melanin autofluorescence evidenced by visibility of choroidal blood vessels (Fig. 4B, upper). The mean fixation location is 6° superior to the anatomic fovea, and a larger circle of 3.1° radius is required to encompass the fixation instability (Fig. 4B, white dot and white circle). Two sets of FPPs obtained on the same day show high concordance (Fig. 4B, lower) with test-retest differences ranging up to 2 dB at individual locations.

Next, we considered all available test locations with a measurable sensitivity ($n = 360$) among *ABCA4*-RD patients ($n = 22$). The TRTSD was 1.52 dB, yielding 95% test-retest limits of ± 4.21 dB for a single test location measured twice. The estimate of TRTSD was almost identical ($P = 0.92$) in *ABCA4*-RD patients with foveal (TRTSD, 1.53 dB; 145 points, 7 patients) and extrafoveal (TRTSD, 1.52 dB; 215 points, 15 patients) fixation. The test-retest variance component from foveally fixating RP patients (102 points, 7 patients) was smaller (TRTSD, 1.15 dB) but not significantly so ($P = 0.495$). The signed test-retest difference was not statistically different from zero ($P = 0.084$), ruling out a major learning effect.

Next we queried whether the overall repeatability coefficient estimated from all test locations in all *ABCA4*-RD patients was a good descriptor of expected variability when patient-specific and locus-specific parameters were considered. In

terms of retinal location, most of the *ABCA4*-RD data clustered in the perifoveal eccentricities, but there was no obvious change in variability as a function of eccentricity from the fovea (Fig. 4C; r^2 relating TRTSD to eccentricity was 2%; $P = 0.529$). In terms of mean thresholds, Bland-Altman plots of test-retest difference against mean sensitivity showed no significant relationship (Fig. 4D; the r^2 relating TRTSD to mean sensitivity was $<1\%$; $P = 0.68$). Next we considered the relationship between variability and local slope (Supplementary Fig. S1A, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8415/-DCSupplemental>) to evaluate whether variability increases at transition zones between regions of normal or near-normal sensitivity and scotomas. A mixed linear model analysis found no effect ($P = 0.914$) of local slope on TRTSD. Among the person-specific parameters was age at the time of testing. With the exception of a fairly large variability in one young patient, no relationship with age was evident on visual inspection (Supplementary Fig. S1B, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8415/-DCSupplemental>), and no correlation was found (including that young patient's observations) between TRTSD and age ($r^2 = 0.02$; $P = 0.593$). Similarly, the r^2 values relating TRTSD to fixation instability (Supplementary Fig. S1C, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8415/-DCSupplemental>) and eccentricity of the fixation location (Supplementary Fig. S1D, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8415/-DCSupplemental>) were 0.01 ($P = 0.72$) and 0.02 ($P = 0.53$), respectively, and indicated no relationship. Results in RP patients (Figs. 4C, 4D, Supplementary Fig. S1A, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8415/-DCSupplemental>) were generally similar to those from *ABCA4*-RD patients.

Variability at the Border of Deep Scotomas

There are at least two important reasons for evaluating variability of microperimetric thresholds near deep scotomas. First, in a clinical trial involving *ABCA4*-RD patients, the expected rate of (centrifugal) expansion of the scotoma boundary may contribute strongly to the design of outcome measures of visual function. Second, previous studies^{46,47} have suggested that the variability of psychophysical thresholds increases near deep scotomas. It is important to note that our analyses (estimating variability as a function of local slope) did not include retinal loci at the immediate boundaries of deep scotomas because, by definition, a value for the slope at these locations is indeterminate. Our FPP test, designed to take advantage of the known boundary at the pBS and the relative parapapillary preservation of vision in most *ABCA4*-RD, afforded the opportunity to evaluate variability as a function of distance from a deep scotoma. First, the eccentricity of the temporal papillary boundary intersecting the FPP was estimated by examining the NIR fundus image obtained during MP1 testing and determining the eccentricity of the first non-detectable threshold location at or near this boundary. The papillary boundary was $13.7^\circ \pm 1.2^\circ$ and $13.7^\circ \pm 1.7^\circ$ eccentric to the fovea for groups of *ABCA4*-RD patients with and without foveal fixation, respectively. Next, MP1 thresholds for each subject were reassigned to an alternative coordinate system with an origin starting at the temporal papillary boundary with increasingly negative eccentricities toward the fovea (Fig. 5). Normal results were analyzed equivalently (papillary boundary at $13.3^\circ \pm 0.8^\circ$), and limits of normal sensitivity were determined along the same alternative coordinate system.

Individual sensitivity profiles in the group of *ABCA4*-RD patients with foveal fixation (Fig. 5A) and with extrafoveal fixation (Fig. 5B) are shown. By definition, there is a steep increase in sensitivity within 1° of the papillary boundary reaching normal limits in the majority of the patients. Test-

FIGURE 4. Test-retest variability along the FPP using retina-tracking microperimetry. (A, B) NIR-RAFI images of P7 and P31 representing ABCA4-RD patients with foveal and extrafoveal fixation, respectively. Overlaid are the fixation loci (black crosses) and the FPP profile (dashed lines). Microperimetric sensitivities obtained for first and second tests are shown in the lower panels. The center of the fixation record and the magnitude of the variation are also shown (white circle and dot). (C, D) Signed test-retest sensitivity differences (second test minus first test) in all ABCA4-RD and RP patients at all available retinal locations plotted as a function of eccentricity from the fovea (C) or as a function of mean sensitivity (D). Horizontal lines: 95% confidence interval of the overall coefficient of variation.

retest differences plotted along the alternative coordinate system allow examination of variability in the vicinity of the deep scotoma formed by the pBS (Fig. 5C). Qualitatively, the overall 95% test-retest limits (Fig. 5C, solid lines) appeared to encompass the data well and corresponded to the ± 2 SD limits calculated at each eccentricity (Fig. 5C, dashed lines). In a mixed linear model analysis, eccentricity from the pBS had no effect on TRTSDs, either when included as a fixed effect ($P = 0.63$) or as a linear covariate ($P = 0.97$). In the model with eccentricity from the pBS included as a linear covariate, the slope relating TRTSD to eccentricity from the pBS was 0.017 (SE, 0.272), which is very close to zero.

DISCUSSION

The pace of clinical trials in hereditary retinal degenerations has been accelerating.^{48–55} For patients with macular degeneration,

early-stage clinical trials of gene and stem cell therapy have already started (www.clinicaltrials.gov, numbers NCT01367444 and NCT01345006), and several more trials are anticipated to start soon. Both early-stage and later stage clinical trials in patients with macular degeneration will have to demonstrate safety and efficacy in terms of a visual function outcome,⁵⁶ but measurement of central visual function with established methods (e.g., visual acuity and conventional perimetry) remains challenging in this population.⁵⁷ The present study included three populations of patients with hereditary retinopathies to evaluate retina-tracking microperimetry as a possible outcome measure in a clinical trial of macular degenerative diseases.

Measuring Macular Function with MP1

There are many choices for measuring some aspect of macular function, depending on the reason for the measurement and

FIGURE 5. Test-retest differences in the immediate vicinity of the pBS. (A, B) Microperimetric sensitivities re-plotted as a function of the distance from the temporal papillary boundary defining the start of the pBS in *ABCA4*-RD patients with (A) or without (B) foveal fixation. (C) Signed test-retest sensitivity differences as a function of eccentricity (toward the fovea) from pBS. 95% confidence interval of the variation estimated at each test locus (*dashed lines*) is similar to the overall coefficient of variation (*solid lines*).

the availability of equipment, resources, and time. When considering drugs or treatments designed to slow down the natural history of a progressive retinopathy that shows a complex spatiotemporal distribution of disease severity, it would be highly desirable to sample vision at many defined retinal loci. Such localized measures of “vision” can be then interpreted with now widely available imaging modalities (e.g., OCT and autofluorescence) providing high-resolution information on localized structural changes of the retina and the RPE. One conventional measure of vision is subjective visibility thresholds of small, short-duration stimuli as performed by automated static perimetry⁵⁸ and a variant of this method measuring dark-adapted function.³² In conventional perimetry, retinal localization of a stimulus is implied indirectly from the assumed retinal location of fixation. This approach can work well when fixation is stable and foveal.⁵⁹ Loss of fixation stability or foveal vision, such as occurs commonly in *ABCA4*-RD, complicates the measurement of macular function with conventional perimetry. Accurate correspondence between retinal structures and visual function requires simultaneous imaging of the fundus, and such methods have been in existence for > 30

years.^{60–67} It is, however, the commercial availability of systems^{14,68} that include real-time video tracking of the fundus and appropriate compensation of the location of stimulus presentation that have allowed more widespread use of automated retina-tracking perimetry to be performed at predefined retinal loci.

What are the appropriate stimulus conditions using an MP1 instrument for assessing *ABCA4*-RD? Most reports in the literature have opted to use Goldmann III-sized stimuli with white flashes on a white background.^{14,16–26,28,29} There are at least two reasons for this choice. First, white-on-white conditions are thought to approximate the conventional achromatic perimetry even though the background level of MP1 is substantially lower than the 10 cd/m² used in Humphrey and Goldmann perimeters. Second, normal values are provided by the manufacturer only for this combination of conditions. However, Goldmann III-sized white-on-white MP1 stimuli demonstrate strong ceiling effects at which the dimmest (20-dB) stimulus is easily visible to most healthy subjects. We chose Goldman III-sized stimuli with red flashes on a red background¹⁰ to avoid ceiling effects. Both the white and the red

backgrounds are within the mesopic range and thus produce results that are a complex function of rod- and cone-driven vision.⁶⁹

What is the appropriate microperimetric test pattern to use to evaluate macular function in *ABCA4*-RD? Most reports in the literature evaluating macular degenerations have used two-dimensional patterns covering the macular region with a $\sim 2^\circ$ point-to-point sampling distance,^{14,15,17,20,21,23,24,27,29} similar to patterns used in conventional perimetry. It is important to note, however, that the use of real-time retina-tracking perimetry theoretically affords much higher spatial density of testing than conventional perimetry. Indeed, the use of the label *microperimetry* implies testing with greater spatial density than conventional perimetry. However, increased sampling density also means increased testing burden because of prolonged testing time; therefore, investigators using higher density patterns have limited the testing to small retinal regions.^{18,19,22} We argued that opposing considerations of high spatial density testing and practical testing times can be balanced by the use of one (or more) unidimensional pattern of wide extent along a meridian chosen appropriate to the disease phenotype under consideration. In *ABCA4*-RD, the advantage of high spatial density sampling includes functional evaluation of transition zones between more diseased central retina and less diseased pericentral retina. Further, with the use of higher spatial density sampling, a statistically significant centrifugal expansion of the transition zone may be detectable over a shorter time compared with a less dense sampling grid. Importantly, our test-retest results in the vicinity of the deep scotoma formed by the ONH suggest that reliable visual function information can be obtained at least within a spatial certainty of 1° .

Repeatability of Visual Function Measurements with MP1

ABCA4-RD is generally a slowly progressive condition. In longitudinal studies performed over an average duration of 8.7 years, we have recently shown that in the peripheral retina, within the subset of patients who already show evidence of peripheral disease at the first visit, visual function is lost at rates of 1.1 dB/year and 0.45 dB/year for rods and cones, respectively.¹² At the most central region we studied (30° eccentricity from the fovea), the combination of centrifugal and retina-wide progression components predict somewhat higher rates of loss in the vicinity of 1.3 dB/year and 0.7 dB/year for rods and cones, respectively.¹² A recent longitudinal (12-month) study in nine patients that included three patients with *ABCA4*-RD suggested a sensitivity loss of 3 dB/year at the edges of *macular* scotomas.²⁹ Whether this substantially higher progression rate is representative of the *macular* disease in the majority of *ABCA4*-RD remains to be evaluated in a larger series of patients. Assuming a natural progression rate can be established, variability of the specific visual function measurement chosen as an outcome in a clinical trial will define the minimum duration that will be necessary to observe patients to detect significant changes.

Our estimate (95% confidence interval) of the point-by-point test-retest limits was 4.2 dB. Likely contributors to this variability are natural fluctuations in perception, attention, and concentration, well known from conventional perimetry. Unlike projection perimetry, retina-tracking microperimetry is not expected to be affected by blinks, saccades, and instantaneous losses of fixation because stimuli are presented only while the retina is being tracked. On the other hand, because of the Maxwellian view setup of microperimetry, a minor amount of clipping by the iris can change the retinal illuminance of the stimulus and the background and can contribute to variability. How does our point-by-point test-retest limits (using Goldmann

III red-on-red stimuli along the FPP in *ABCA4*-RD patients) compare to the literature? To our knowledge, there is only one other estimate of pointwise MP1 variability in the current literature. The authors report²⁴ test-retest limits of 5.56 dB after censoring the ceiling effect due to the Goldmann III white-on-white stimulus conditions used.^{16,21,24,25,29,41–43} The higher variability observed compared with our results could be partially attributed to the different patient populations used; a large number of AMD patients were included in that study,²⁴ unlike our cohort of relatively younger *ABCA4*-RD patients.

Our estimate of microperimetric variability did not show a relationship with mean sensitivity in *ABCA4*-RD (or in RP). These results imply that detectability of significant visual function changes is independent of the local severity of degeneration (as long as there is measurable function). Our current results are comparable to the lack of a relationship we previously found in a group of 35 RP patients tested under dark-adapted conditions.⁷⁰ Further supporting a lack of relationship is a comparable pointwise test-retest variability found in *RPE65*-LCA patients even though they demonstrate 40 dB or greater losses of sensitivity on average and thus form an extreme case of sensitivity loss.⁵¹ Other investigators, however, have found a complex relationship between variability and sensitivity in RP patients tested under light-adapted conditions⁷¹ more reminiscent of findings in glaucoma and optic neuritis.^{72–74} A component of the differences found in variability of RP patients^{70,71} may be due to differences in the inclusion of transition zones between healthier and diseased retinas. With conventional perimetry, variability increases at transition zones,^{46,47,75} which are exactly the retinal regions expected to show the greatest rate of disease progression in *ABCA4*-RD.²⁹ Unlike conventional perimetry, we show that variability is not related to the local slope of sensitivity in *ABCA4*-RD when using retina-tracking microperimetry (Supplementary Fig. S1A, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8415/-DCSupplemental>). Even more important, there was no increase in variability within 1° of the absolute scotoma formed by the physiological blind spot (Fig. 5). Thus we conclude that the overall estimate of repeatability is applicable to transition zones as well as homogeneous zones under the testing conditions described here.

Microperimetry as a Macular Function Outcome Measure in Clinical Trials

The pathophysiology of *ABCA4*-RD is thought to include abnormal clearance of bisretinoid compounds in the photoreceptors and the RPE, with resultant anatomic and functional changes progressing with centrifugal (center to periphery) and retina-wide components over many decades.^{8,10,12} The peripapillary region is relatively preserved.^{9,10,44,45} The macular retina is consistently involved in all stages of *ABCA4*-RD, and new technologies of autofluorescence, cross-sectional imaging, and adaptive optics are providing increasingly detailed information on the anatomic/structural changes occurring at the level of photoreceptors and the RPE of the macula.^{10,28,76,77} Such findings can sometimes be used as surrogate outcomes for visual function in clinical trials⁵⁶ but do not replace visual function. In recent clinical trials, for example, substantial changes in visual function in the short⁵¹ or longer³⁰ term were not associated with changes in structural outcomes, thus emphasizing the importance of direct measures of vision. Furthermore, visual function abnormalities do not always correlate with structural findings.⁷⁸ Our work took on the challenge of measuring macular function in macular degenerations, and our results suggest that retina-tracking microperimetry can be used to measure macular function at specific retinal locations with a predictable and acceptable reliability.

Acknowledgments

The authors thank Elaine Smilko, Alejandro Roman, Elizabeth Windsor, and Alexander Sumaroka for patient coordination, data acquisition, and analyses.

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