Evaluating visual field (VF) defects is important for clinical diagnosis and monitoring of various ophthalmologic diseases. VF is assessed mainly by subjective perimetry techniques, including standard automated perimetry and short-wavelength automated perimetry.1 Two insurmountable limitations of these methods are the need for patient cooperation and the subjectivity of patients’ responses.2 Therefore, testing of young children, the elderly, and individuals with compromised communication is almost certain to yield unreliable results. Moreover, patients’ responses can be affected by their levels of fatigue, wakefulness, and attentiveness during the long procedure. Hence, constant monitoring and instruction of participants by suitably qualified personnel are needed to obtain reliable results. Furthermore, test–retest variability, particularly in peripheral locations and in regions of VF deficits, makes it difficult to determine whether the VF is worsening over the course of serial examinations.1,3–5 Frequent examinations are needed and misdiagnosis of early stages is common.6–8

Unfortunately, in routine clinical practice the frequency of VF examinations varies considerably, further emphasizing the need for new technological advances that allow earlier and objective detection of VF defects and their progression with higher levels of certainty than are currently available.9

The pupillary light reflex is an objective indicator of retinal and optic nerve functions.10–15 Several studies used a pupillometer with achromatic stimulus for objective determination of the visual field.9,12–23 However, a comparison between visual and papillary sensitivity revealed that they are not sufficiently correlated to be of clinical use.9,14–23 A different method of multifocal pupillographic perimetry (TrueField Analyzer; Tektronix, Beaverton, OR) uses white and colored stimuli analyzing both eyes simultaneously.24–26 Although this technology is promising, it cannot differentiate between the rod and cone systems.

Kardon et al.,27,28 using full-field stimuli, developed a protocol for assessing the contribution of rods, cones, and melanopsin ganglion cells to the pupillary response (PR). These studies provided evidence that the PR to different wavelengths,
stimulus intensities, and stimulus durations reflects activation of different outer and inner retinal cells. It was suggested that the transient PR to a low-intensity, short-wavelength stimulus reflects rod activity, that the transient PR to a long-wavelength stimulus is predominantly driven by cones, and that a sustained PR to a continuous high-intensity short-wavelength stimulus is derived primarily from the direct intrinsic activation of melanopsin-containing retinal ganglion cells (mRGCs).27–29 These and similar protocols were successfully used to assess the function of outer and inner retinal cells in patients with retinitis pigmentosa (RP) and patients with RPE65 mutations.30,31 However, because these methods use a wide light source that stimulates the entire retina, they are not applicable for multifocal testing to identify VF defects.

In a previous study we demonstrated that a modified Goldmann dark-adapted chromatic perimeter can be used to identify cone or rod VF defects.32 Here, we tested the possibility of using a novel chromatic perimeter technique in which the retina was stimulated in a multifocal pattern by using a narrow (64 mm²) light beam at different wavelengths. Use of this narrow beam resulted in generation of an objective VF test. The PR in this modified system (dark-adaptometer; Roland Consult Stasche & Finger GmbH, Brandenburg, Germany) was automatically recorded at various VF locations. We compared between normal participants and patients with RP or cone–rod dysfunction. We also associated the chromatic pupillometer-based perimeter findings of patients with their electroretinography (ERG), and dark-adapted chromatic Goldmann perimeter recordings.

METHODS

Participants

The study was conducted according to the tenets of the Declaration of Helsinki, received approval from the Sheba Medical Center Institutional Review Board Committee, and was registered at www.clinicaltrials.gov (registration no. NCT01021982). Informed written consent was obtained from all participants. Twenty eyes of 12 normal healthy age-matched (P = 0.067 compared with patients) volunteers (six males, six females; mean ± SD age: 38 ± 14.4 years; range: 25–65 years) were included in the study. Four participants could not have both eyes tested. Inclusion criteria were normal eye examination, best-corrected visual acuity (BCVA) of 20/20, normal color vision test (Roth-24-hue test), no history of past or present ocular disease, no use of any topical or systemic medications that could adversely influence efferent pupillary movements, and normal 24-2 Swedish Interactive Threshold Algorithm (SITA), developed for the Humphrey standard perimeter (Humphrey Field Analyser II, SITA 24-2; Carl Zeiss Meditec, Inc., Jena, Germany).

The study group (eight males and nine females; mean ± SD age: 48.8 ± 15.5 years; range: 27–72 years) comprised 30 eyes of 16 patients with RP and two eyes of a patient with cone–rod dystrophy. Inclusion criteria for RP patients were typical abnormal fundus appearance and a previously recorded ERG that was abnormal under scotopic or photopic conditions or both (in compliance with the protocol of the International Society for Clinical Electrophysiology of Vision, which specifies the absence or diminution of b-wave amplitude below the fifth percentile with prolonged implicit times compared with normal participants).33 Exclusion criteria were a concurrent ocular disease and any other condition affecting the PR. Data recorded for all patients included sex, diagnosis or genetic defect if known, and ERG responses.

Light Stimuli

Light stimuli were presented using a ganzfeld dome apparatus (multifocal dark-adaptometer; Roland Consult Stasche & Finger GmbH) placed 330 mm from the patient’s eye, and controlled with a stimulus generator and custom software. The untested eye was occluded. Stimuli were presented from the center, and participants were asked to fixate on a red light-emitting diode fixation light presented from 13 different locations in the VF (central, superior, inferior, temporal, and nasal fields at angles of 10°, 20°, and 30°). Wavelengths of the light stimuli selected for this study were 640 ± 5 nm for red light (long wavelength) and 480 ± 5 nm for blue light (short wavelength). A light intensity of 40 cd/m² was chosen after preliminary calibrations that enabled us to identify the minimal stimulus intensity that yielded a substantial PR in peripheral VF locations in five normal participants. Each stimulus was presented using stimulus size V (64 mm²) on a background luminance of 2.7 cd/m². Stimulus duration was 1000 ms and the interstimulus interval was 10 seconds.

Pupil Measurement

Pupil diameters were recorded in real time by a computerized infrared pupillometer (Roland Consult Stasche & Finger GmbH), which consisted of a monitor with viewing optics for presentation of a light stimulus to the subject. Pupil tracking was performed by an infrared high-resolution camera inside the dark-adaptometer that recorded the PR at a sampling rate of 34 Hz. The software (Roland Consult Stasche & Finger GmbH) searched for the pupil in every image. A correction factor was used to get the diameter in millimeters and pupil diameters were measured with an accuracy of 0.1 mm (Roland Consult Stasche & Finger GmbH).

The subject’s eye was inclined at 15° to the center, at the position where the stimulus was presented (Fig. 1). The subject had an uninterrupted VF in excess of 30° in all meridians. A recordable PR was obtained in both eyes of all patients except for two, both of whom had difficulty in fixating on most fixation locations in one eye.

The subjects were requested to blink several times before the start of the recording and refrain from blinking during the recording. Real-time video imaging of the eye was carefully monitored by the examiner during the test. Tests in which the subject blinked were excluded and the subject was retested.

Analysis of Pupillary Responses

Percentage pupil contraction at each time point was determined by the formula: % pupil contraction = 100 × [The difference between the highest initial diameter at the beginning of the stimulus and the lowest diameter in response to that stimulus]/[The highest initial pupil diameter], as described by Kardon et al.27 Previous studies demonstrated that contraction of the pupil is a true PR when the initial pupillary contraction (time at which the maximum acceleration occurs) falls within a definite time window (200–450 ms after stimulus onset).13,27 Accordingly, we recorded pupillary contraction only when the initial pupillary contraction was within this time window. Figure 1 shows an example of a pupil tracing from a normal subject. All calculations were done by an independent experienced masked technician. The test duration was approximately 5 minutes for each eye. In preliminary studies we repeated each measurement twice in normal participants and found no significant difference between repeated measurements (P > 0.05, n = 14). Furthermore, in all perimeter locations the PR did not significantly differ.
between the left and right eye \( (P > 0.16, n = 7) \) in the healthy participant.

**Chromatic Dark-Adapted Visual Field**

Eleven patients were tested for kinetic VF by dark-adapted chromatic Goldmann perimetry. Briefly, a Goldmann perimeter (940-ST; Haag-Streit AG, Liebefeld, Switzerland) was used to map patient’s conventional and two-color dark-adapted VFs. Patients were dark adapted for 30 minutes prior to testing. The setting used for stimuli were \( V_{3c} \) for the long-wavelength stimulus and 2 log units lower in luminance \( (V_{3c}) \) for the short-wavelength stimulus.

**Statistics**

Statistical analysis was performed using a commercial software program (SAS for Windows, version 9.2; SAS Institute, Inc., Cary, NC; or SPSS for Windows, version 20.0; SPSS, Inc., Chicago, IL). For two-eye analysis, comparison between patients and healthy controls for all perimetry locations was performed using a one-way ANOVA with repeated measures (eye side). Since for some of the participants only one eye was examined, the mixed model was applied to address this issue. For single-eye analysis, we compared between the pupillary recordings of the right eye of patients and healthy controls for all perimetry locations using a one-way ANOVA. Agreement between the chromatic pupillometer recordings and the dark-adapted chromatic Goldmann (that yields a yes/no result) was assessed using two-sample t-test and the Mann–Whitney nonparametric test. A value of \( P < 0.05 \) was considered statistically significant.

**RESULTS**

All participants easily tolerated the protocol without any discomfort. PR to the short-wavelength stimulus significantly exceeded the PR to the long-wavelength stimulus at all perimetry locations \( (P < 0.01) \). Thus, the mean percentage pupil contraction in the normal participants in response to the short-wavelength stimulus was \( \text{mean} \pm \text{SE} \) 28.6 \( \pm \) 0.38, and only 14.7 \( \pm \) 0.41% in response to the long-wavelength stimulus (Figs. 2A, 2B).

When both eyes were included in the analysis (repeated-measures ANOVA), no significant differences in mean PR to the long-wavelength stimulus were observed between RP patients and normal participants in the majority of locations \( (P > 0.05) \), except nasal at 20°, temporal at 30°, and superior at 30° \( (P \leq 0.02, \text{Fig. 2A}) \). Similar results were obtained when the pupillary responses of one eye of patients and normal participants were compared. Thus, the mean PR to the long-wavelength stimulus
in RP patients was significantly lower compared with normal participants in only two locations (nasal 20° and temporal 30°, \(P < 0.04\), Fig. 3A).

In contrast, the mean PR to the short-wavelength stimulus exhibited by the RP patients was significantly lower than the PR of control participants in all locations (\(P < 0.03\)), except temporal 10°, when both eyes were analyzed (Fig. 2B). When only one eye was included in the analysis, the mean PR to the short-wavelength stimulus exhibited by the RP patients was significantly lower than the PR of control participants in a majority of locations (\(P < 0.011\)) except temporal 10° and superior at 10° and 20° (Fig. 3B). The lowest responses by RP patients were consistently recorded in peripheral locations (20° and 30°).

To validate the novel chromatic pupillometer-based perimetry technique we compared the chromatic pupillometer recordings of 11 patients with their chromatic dark-adapted Goldmann findings. In a majority of locations the chromatic pupillometer recordings of PR to short-wavelength stimulus were in agreement with the chromatic Goldmann findings both when single-eye and two-eye analyses were performed (\(P < 0.05\); Supplementary Table S4). By contrast, in a majority of locations there was no significant correlation between the PR to long-wavelength stimulus and the chromatic Goldmann recordings (Supplementary Table S3). In all patients tested, minimal pupillary responses were recorded in areas that were nondetected in the dark-adapted chromatic Goldmann (Supplementary Tables S1–S4). To illustrate the pattern of recorded PR values compared with the results of chromatic Goldmann VF testing, the individual reports on three patients, two with RP and one with cone–rod dystrophy, are presented here.

RP Patient #1
A 68-year-old white male with isolated RP had a BCVA of 20/30 in both eyes. Fundus examination demonstrated pigment epithelial atrophy, arteriolar narrowing, and bone spicules and clumps in the midperiphery of both eyes (the right eye is shown in Fig. 4A). This patient had significant ERG and VF loss (Table and Fig. 4B). The PR of the right eye of this patient to both the long- and the short-wavelength stimuli in a majority of VF locations, was lower by over 3-fold compared with the mean PR of the right eye in the normal group (Fig. 4C). In most of the tested perimetry locations there was an agreement between the pupillometer-based perimetry and the chromatic Goldmann perimetry. Thus, the pupillometer-based perimetry showed that the PR to the long-wavelength stimulus was highest at temporal 20° and superior 30° (Fig. 4C). These locations corresponded to the areas where the long-wavelength stimulus was detectable by the chromatic Goldmann perimetry (Fig. 4A). In areas where the long-wavelength stimulus was not detected by the chromatic Goldmann (10° superior, and all inferior and nasal locations), the PR response was lower than 15% of mean normal values. Similarly, the highest pupillary responses to the short-wavelength stimulus were recorded at 30° superior and at 10° and 20° temporal, in agreement with the areas where the short-wavelength stimulus was detected by chromatic Goldmann (Figs. 4B, 4C). The lowest PR to the short-wavelength stimulus (<10% of normal
mean values) was obtained in locations that were not detected by the chromatic Goldmann (center and all nasal locations). Lower correspondence was observed in areas that were on the isopters of the chromatic Goldmann VF (temporal 10° and superior 20° for both wavelength stimuli).

**RP Patient #2**

A 37-year-old white male with autosomal dominant RP had a BCVA of 20/20 in both eyes. Fundus examination demonstrated pigment epithelial atrophy, arteriolar narrowing, and bone spicules in his right eye (Fig. 5A). ERG responses were abnormal (Table) and chromatic Goldmann perimetry demonstrated characteristic constriction (Fig. 5B). In most of the locations tested there was an agreement between the pupillometer-based perimetry and the chromatic Goldmann perimetry. Thus, the PR to the long-wavelength stimulus (Fig. 5C) was 75% or higher of the mean response of the right eye in normal participants at the center, at 10° and 20° in all locations, and at 30° nasal. These locations corresponded to areas where the long-wavelength stimulus was detectable on chromatic Goldmann perimetry (Fig. 5B). By contrast, in areas where the long-wavelength stimulus was nondetectable by the chromatic Goldmann or close to the isopter (30° temporal, inferior, and superior), minimal pupilary responses were recorded (54%, 60%, and 28% of mean normal values, respectively). Similar agreement was observed in the PR to the short-wavelength stimulus. In areas where the short-wavelength stimulus was detectable by chromatic Goldmann perimetry (at the center, all nasal locations, inferior 10° and 20°, and superior 10° and 20°), the recorded PR was...
maximal (>66% of mean normal values). The lowest pupillary responses were recorded in areas where the short-wavelength stimulus was not detectable on chromatic Goldmann perimetry (temporal 20\(^8\), superior 30\(^8\), and inferior 30\(^8\)). Lower correspondence was observed in nasal 30\(^8\) for the short-wavelength stimulus, which was on the isopter of the chromatic Goldmann VF.

**Cone–Rod Dystrophy Patient #17**

A 42-year-old male with autosomal recessive cone–rod dystrophy had a BCVA of 20/100 at both eyes. Fundus examination demonstrated macular pigment epithelial atrophy (the right eye is shown in Fig. 6A) and chromatic Goldmann perimetry revealed a dense central scotoma and temporal parafoveal shifting of fixation (Fig. 6B). ERG responses were abnormally reduced, especially under conditions of light adaptation (Table). In his responses to both the short- and the long-wavelength stimuli, this patient demonstrated an agreement in most perimetry locations between the pupil-meter-based perimetry and the chromatic Goldmann perimetry (center, 10\(^8\), superior 30\(^8\), and inferior 30\(^8\)). Lower correlation was observed in nasal 30\(^8\) that is on the isopter and at 30\(^8\) temporal for the short-wavelength stimulus.

**DISCUSSION**

In this study we successfully used the PR to multifocal chromatic stimulus as an objective mean to perform perimetry. RP patients exhibited significantly reduced mean PR to the short-wavelength stimulus at most locations, whereas their mean responses to the long-wavelength stimulus were similar to those of normal participants at central locations but were significantly reduced at peripheral locations. Studies from the groups of Kardon\(^27\)–29 and Stieger\(^30\) demonstrated that transient PR to a low-intensity, short-wavelength stimulus reflects rod activity and that transient PR to a long-wavelength stimulus is predominantly driven by cones. In retinitis pigmentosa patients, the loss of rod function exceeds the reduction of cone function and VF loss typically begins with peripheral VF constriction.\(^35\),\(^36\) These findings suggest that the PR to short-wavelength stimulus measured by our chromatic pupillometer may be mediated by rods, whereas the PR to long-wavelength stimulus measured here may be mediated by cones. Future studies will be aimed at determining a clinically applicable protocol for assessing these cell contributions to the PR measured by our chromatic pupillometer. This will enable both the objective detection of affected areas and the identification of the damaged photoreceptor cells underlying the defect in these locations.

When both eyes were analyzed, the only measurement in which the PR to short-wavelength stimulus in RP patients was not significantly lower than that in normal participants was at the temporal 10\(^8\) location. This might be explained by recent optical coherence tomographic findings in RP patients showing increased outer macular thickness in the nasal quadrant, which corresponds to the temporal 10\(^8\) location.\(^37\) In cone–rod dystrophy, the deficit in the cones far exceeds that in rods. In the cone–rod dystrophy patient described here, the decline in PR to both the long- and the short-wavelength...
stimuli was similar in the scotoma area, unlike our RP patients, in whom the decline in response to the short-wavelength stimulus was more pronounced, suggesting that the new perimetry method may assist in diagnosis of diseases affecting different retinal cells.

To validate the new chromatic perimetry technique we decided to compare the novel pupillometer method with an established perimetry technique. We chose the chromatic dark-adapted Goldmann because it uses multifocal chromatic short- and long-wavelength stimuli for perimetry determination and monitoring of patients with retinal dystrophies.\(^3^2,^3^8\) We observed a good agreement between the chromatic pupillometer-based perimetry and the malfunctioning areas identified by the dark-adapted chromatic Goldmann perimetry. Furthermore, a correlation was observed between the two methods in a majority of locations in response to the short-wavelength stimulus. Our findings that the PR to a long-wavelength stimulus did not correlate in a majority of locations with the chromatic dark-adapted Goldmann, could be explained by the differences between the two methods: the Goldmann is a threshold-subjective kinetic test that yields a binary qualitative recording (yes/no result). By contrast the chromatic pupillometer is a suprathreshold, objective quantitative test. Hence, it is likely that the pupillometer can detect reduced retinal function to suprathreshold stimuli in a numeric manner. Since the response to the long-wavelength stimulus was less affected in RP patients, this difference between the two systems was more profound. In some cases the lower agreement could be explained by PR measurements in areas corresponding to the chromatic Goldmann VF isopters.

Our findings that similar results were obtained using two-eye and single-eye analyses provide further evidence for the validity of the new perimetry method. Minor differences between the results of the two analyses could be explained by the smaller sample size using a single-eye analysis. Although the results presented here are highly promising for use of the multifocal chromatic PR as an objective parameter of retinal function in disease conditions, further research is needed to improve and refine this technique. The prototype instrument used in this study was constructed for proof of concept and
uses only a single central stimulus. Thus, some minimal cooperation on the part of the participants was still needed for fixation on targets. In some cases, the need for a patient to fixate on a location correlating with a scotoma could explain a reduced correspondence between the pupillometry-based perimetry and the Goldmann perimetry findings. For example, in the cone–rod dystrophy patient, who had difficulty fixating at central locations because of a central scotoma, we found relatively less correspondence between the Goldmann and the pupillometry-based test results, specifically in more peripheral locations. We believe that this reduced correspondence may be due to the central location of the stimulus and the limited ability of this patient to fixate on peripheral VF locations using parafoveal fixation. This limitation is likely to be overcome in a future design of the instrument, where participants will be asked to look forward and stimuli will be individually introduced at different VF locations. A second limitation of the current study was the use of the short-wavelength stimulus at 40 cd/m². Based on the findings of Kardon et al., this light intensity can exert a pupillary response both in rods and in mRGCs. However, since transient PR was recorded, the contribution of rods probably exceeded that of mRGCs. Furthermore, the pathology of RP patients is caused primarily by degeneration of the photoreceptors, and the defect in mRGCs is less significant, suggesting that the difference in PR to the short-wavelength stimulus between the RP patients and the normal participants was largely due to rod degeneration. In an attempt to discriminate between responses of mRGCs and the rods, we are currently testing the PR to short-wavelength stimuli at different intensities. Once a protocol for differential cell-type contribution to PR is established, this chromatic pupillometer will enable determination of the functionality of inner and outer retinal cells at different locations in the retina. Areas with nonfunctional photoreceptors and some functional mRGCs may be more suitable candidates for cell-based or genetic therapies. In future studies we will also examine the correlation between age and severity of VF loss in a larger group of patients as well as determine the appropriate parameters for testing the PR of dichromats. In addition, use of a smaller spot size will be investigated, with the aim of

**FIGURE 6.** A 42-year-old male with autosomal recessive cone–rod dystrophy. (A) Color photograph of the right eye shows an abnormal fundus with macular pigment epithelial atrophy. (B) Chromatic Goldmann perimetry of the right eye. (C) Comparison of PR of the patient’s right eye in response to both short- and long-wavelength stimuli, as a percentage of mean normal values. Visual field locations are marked as described in Figure 2.
achieving better perimetric resolution. Taken together, this novel chromatic pupillometer may enable sensitive and objective characterization of VF defects and may be used to diagnose and monitor patients with photoreceptor dystrophies in the upcoming clinical trials.

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