A Quantitative and Qualitative Exploration of Photoaversion in Achromatopsia

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Purpose. Photoaversion (PA) is a disabling and ubiquitous feature of achromatopsia (ACHM). We aimed to help define the characteristics of this important symptom, and present the first published assessment of its impact on patients' lives, as well as quantitative and qualitative PA assessments.

Methods. Molecularly confirmed ACHM subjects were assessed for PA using four tasks: structured survey of patient experience, novel quantitative subjective measurement of PA, visual acuities in differing ambient lighting, and objective palpebral aperture-related PA testing.

Results. Photoaversion in ACHM was found to be the most significant symptom for a substantial proportion (38%) of patients. A novel subjective PA measurement technique was developed and demonstrated fidelity with more invasive paradigms without exposing often very photosensitive patients to brighter light intensities used elsewhere. An objective PA measurement was also refined for use in trials, indicating that higher light intensities than previously published are likely to be needed. Monocular testing, as required for trials, was also validated for the first time.

Conclusions. This study offers new insights into PA in ACHM. It provides the first structured evidence of the great significance of this symptom to patients, suggesting that PA should be considered as an additional outcome measure in therapeutic trials. It also offers new insights into the characteristics of PA in ACHM, and describes both subjective and objective measures of PA that could be employed in clinical trials.

Keywords: achromatopsia, photoaversion, photophobia, gene therapy, lightness/brightness perception

Achromatopsia (ACHM) is an autosomal recessive condition affecting 1 in 30,000 people[1] associated with a lack of cone photoreceptor function.[2] It is characterized by presentation at birth/early infancy with pendular nystagmus, poor visual acuity (VA; approximately logMAR 1.0), a lack of color vision, and marked photophobia/hemeralopia. To date, six genes have been associated with ACHM (*CNGA3*,[3] *CNGB3*,[4] *GNAT2*,[5] *PDE6C*,[6] *PDE6H*,[7] and *ATF6*[8]), with all except the lattermost encoding components of the cone-specific phototransduction cascade. Disease-causing variants in *CNGA3* and *CNGB3* account for about 80% of cases.[9–12]

Several studies have demonstrated effectiveness of gene-based therapy to restore cone function in animal models of ACHM,[13–16] resulting in the commencement of phase I/II human gene replacement trials (ClinicalTrials.gov identifiers: NCT02599922, NCT03001310, NCT02610582, NCT02935517). A phase I/II study using ciliary neurotrophic factor (CNTF) in *CNGB3*-associated ACHM[17] showed no improvement in photopic ERG responses, VA, or color discrimination, but reported reduced sensitivity to bright lights in treated eyes.

Discomfort and/or avoidance of bright lights is a classical feature of ACHM.[1] The terminology to describe this phenomenon is varied, and its usage can be inconsistent. Photoaversion (PA) is the avoidance of light due to discomfort and or/impaired VA, whereas photophobia and photo-oculodynia are the sensation of pain from a normally non-noxious light source. Hemeralopia refers to reduced VA in photopic ambient viewing conditions. Despite the ubiquitous nature of this symptom in ACHM, there is limited associated literature.

A reduction in achromat VA with increasing luminance has been documented previously,[18] and a few case reports have suggested that photophobia may be one of the more debilitating symptoms.[19–21] Recently, Zelinger et al.[22] described measurements of PA made in three *CNGA3* ACHM patients. They assessed PA by measuring the percentage change in palpebral aperture under different lighting conditions. They reported that, when lighting conditions changed abruptly from complete darkness to dim light, normal subjects' palpebral apertures did not differ, but that the three *CNGA3* patients tested showed an approximately 50% reduction. However, there remains to date no evidence base in the literature as to which of the aspects of PA (e.g., discomfort or reduced vision) is most bothersome to ACHM patients, nor has there been any structured assessment of patient-reported experiences of PA and its effect on their daily living. Given the potential therapeutic effect on this symptom by human interventional trials, this study attempts to provide some further data on this topic.

Patients in the aforementioned *CNGB3* human CNTF treatment trial[17] reported that treated eyes were less sensitive to light than their untreated eyes; this diminished photophobia was postulated to be due to reduced rod sensitivity, given that CNTF downregulates rod phototransduction. If reduced photophobia is going to be one of the benefits of intervention, then more objective measures of this albeit subjective symptom will be needed moving forward. Moreover, the importance of assessing what a patient experiences and might want from future treatments is increasingly recognized in global healthcare structures, such as the UK's National Health Service (NHS).

The proposed mechanisms of photophobia are multiple and not fully understood.[23] The relative effect of various properties of light has been investigated in other patient groups who suffer from PA, but not in ACHM. Migraineurs have been found to have lower discomfort thresholds than controls for shorter wavelength (SW, blue) and long wavelength (LW, red) light compared to middle wavelength (green) light.[24] Stringham et al.[25] showed that normal subjects' increasing PA (as measured by electromyography [EMG] of the squinting response) correlated with decreasing stimulus wavelength, which they postulated acts as a mechanism biased to protect against SW light.

The greater sensitivity to SW light may relate to the discovery of a new class of photoreceptor: melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs), which detect nonimage forming light and constitute 1% to 3% of the GC population.[23,26,27] Intrinsically photosensitive RGCs have been implicated as mediators of behavioral functions including circadian rhythm regulation, mood and migraine-associated photophobia,[28] and are more sensitive to SW light.[29] Given that both rod and cone input modulates ipRGC output,[28] the absence of functional cones in ACHM might be expected to affect output from ipRGCs, either directly, or via a bystander effect on rods due to absence of cones.

The effect of stimulus location has also been examined in normals, demonstrating an increase in PA thresholds (as measured by EMG) with retinal eccentricity[30]; these authors propose that PA in normals is thus biased to prevent the central retina from exposure to intense light.

Notwithstanding these studies, there is a general paucity of data in the literature regarding measurement of PA. This may relate to the inherent difficulty in measuring such a subjective phenomenon as discomfort or pain. A PubMed search (March 25, 2017) for any of the terms in the title ("photoaversion," "photophobia," "photo-oculodynia," or "hemeralopia") returned a total of only just over 100 articles in the last decade; the majority from the migraine literature, with only about 20 including retinal causes.

Previously described measurements of bright light intolerance or photophobia in conditions such as migraine or blepharospasm, where light intensities are increased until evident discomfort is induced, are likely to be unsuitable in ACHM, given these patients often experience exquisite photophobia. For example, methods previously used have increased light intensity up to 23,500 lux[31]—which is approximately equivalent to the upper range of full daylight—until a frank sensation of discomfort was reached. Aguilar et al.[32] used a paradigm of self-reporting of photophobia thresholds with increasing light intensity from a light-emitting diode array in their comparison of 7 ACHM subjects with 11 normals, and found that the 2 groups had significantly different photosensitivity thresholds (0.75 ± 0.66 log[lux] and 3.32 ± 0.66 log[lux], respectively; $P < 0.001$).

A testing paradigm for PA in ACHM is therefore required that uses sufficiently dim lighting to not cause undue discomfort to patients, especially given they may need to undergo these tests on a repeated basis in the setting of a therapeutic trial.

Methods

The assessment of PA in ACHM was subdivided into four tasks: (1) a structured patient survey (to obtain subjective patient information about the effect of this inherently subjective phenomenon on patients' lives); (2) a quantitative subjective measurement of PA (in order to try and quantify the subjective experience of PA using a standardized method); (3) a functional measure of VA in different ambient light settings; and (4) an objective measure of palpebral aperture narrowing with PA, as adapted and developed from Zelinger et al.[22] for use in therapeutic trials.

The task protocols adhered to the Tenets of the Declaration of Helsinki, and were approved by the Moorfields Eye Hospital Ethics Committee. Informed consent was obtained from all subjects before entering the study. All patients tested had a molecularly proven diagnosis of ACHM.

Structured Patient Survey

A structured survey (Supplementary Material) was devised in order to obtain subjective information about how PA affects the lives of ACHM patients and their activities of daily living (ADLs). To the best of our knowledge, there has been no structured assessment of the effect of PA in ACHM from the patient's point of view reported in the literature previously (PubMed search March 25, 2017; title keywords: "achromatopsia," "rod monochromatism," "photoaversion," "photophobia"). The survey consisted of 13 questions, in a combination of multiple choice; open (free text); and closed structured questions. This survey was developed and adapted specifically for ACHM from a previously published survey designed for photophobia in benign essential blepharospasm.[31]

Quantitative Subjective Measurement of PA Task

Thresholds of EMG-derived photophobia have been shown to have a high correlation with subjects' ratings of threshold photophobia.[25] Given this correlation, a 0 to 100 psychometric scale rating was employed so that subjects could rate the subjective sensation of viewing presented stimuli on a relative comfort scale, where 0 was no PA at all and 100 was a sensation of PA that the subject felt they could not tolerate and would want to look away from the display screen. Finer scaling, such as that employed here, has been shown to substantially enhance the ability to detect smaller degrees of subjective change.[33]

Patients were advised that no stimuli would be expected to cause pain, and that all stimuli were presented on a standard, commercially available PC monitor. This was important because the majority of ACHM patients who were asked to take part explicitly asked not to be exposed to any "bright lights" due to their PA. Different stimuli were used in order to explore the potential PA-inducing attributes of various qualities of the stimulus, such as intensity, size, location, and color. We tested 26 normals and 26 ACHM subjects.

A commercial spectrophotometer (Konica Minolta Chroma Meter CS100A; Konica Minolta, Osaka, Japan) was used to measure the luminance of the stimuli as presented on a dedicated 58.4-cm monitor display (S2340L; Dell, Inc., Round Rock, TX, USA) generated using a slideshow program (PowerPoint; Microsoft Corp., Redmond, WA, USA) on a laptop PC (Inspiron 5000 series; Dell, Inc.) via a high-definition multimedia interface cable. The monitor had the following settings that

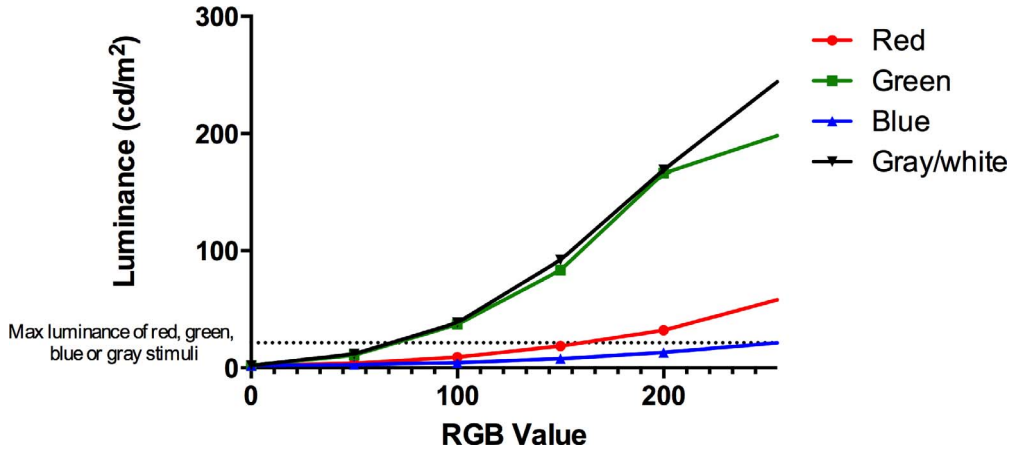

FIGURE 1. Spectrophotometry RGB monitor initial stimuli calibration. Interpolation of the luminance recordings taken for each of the red, green, blue and gray/white monitor stimuli at various RGB values (max 255). This plot allowed the setting of the RGB values in order to maintain isoluminance across all colors (limited by blue at 255).

were maintained at all times: resolution, 1920 × 1080 at 60Hz; preset mode, standard; brightness, 100; contrast, 100; color settings, RGB display; sharpness, 50.

Stimuli for Quantitative Subjective Measurement of PA Task. In order to maintain the isoluminance of the colored stimuli to that of the dimmer (gray) stimuli, measurements of the luminance of the monitor display were taken at various RGB values for each of the red, green, blue, and gray/white stimuli and plotted in Figure 1.

The maximum nonwhite stimulus luminance of 21 cd/m^2 was limited by the maximal luminance of the blue stimulus (see the dotted line in Fig. 1), and the approximate R (red stimulus); G (green stimulus); and RGB (gray stimulus) values were then interpolated from this graph. Spectrophotometer measurements on the stimuli were then made in experimental conditions to fine-tune the interpolated RGB values to all, given a measured luminance of 21 cd/m^2 for all nonwhite stimuli. The RGB values required to do this were as follows: red stimulus, R = 155, G = 0, B = 0; blue stimulus, R = 0, G = 73, B = 0; green stimulus, R = 0, G = 0, B = 255; gray stimulus, R = 71, G = 71, B = 71. These RGB settings displayed stimuli with x, y chromaticity coordinates as measured by the spectrophotometer as follows: white/gray, $x = 0.331$, $y = 0.349$; red, $x = 0.616$, $y = 0.338$; green, $x = 0.316$, $y = 0.559$; and blue $x = 0.161$, $y = 0.081$. A spectroradiometer (Radoma GS-1240; Gamma Scientific, San Diego, CA, USA) was used to measure the peak wavelength of the colored stimuli, and gave measured peak wavelength values of red, green, and blue as 615, 538, and 447 nm, respectively.

The room in which the experiment was undertaken was illuminated by an incandescent 40-W corner stand-lamp, creating a subdued ambient illumination, in order to prevent dark adaptation. All illuminance/luminance measures were taken at the plane of the patient's eyes. The monitor was left on, displaying the large bright white circle calibration stimulus for 20 minutes before any measurements or testing. The monitor displayed bright white stimuli of 250 cd/m^2 luminance, and dimmer gray or colored (red, green, or blue) stimuli of 21 cd/m^2 luminance. The brighter stimuli luminance equaled the luminance of the ETDRS lightbox charts with ambient room lighting on, at which many ACHM patients had previously reported PA anecdotally during routine VA measurements. The black background screen on the monitor (luminance 1 cd/m^2) had an illuminance of 4.2 lux in the ambient lighting. Figure 2 shows the 20 stimuli that were randomly presented during each of the three trial runs.

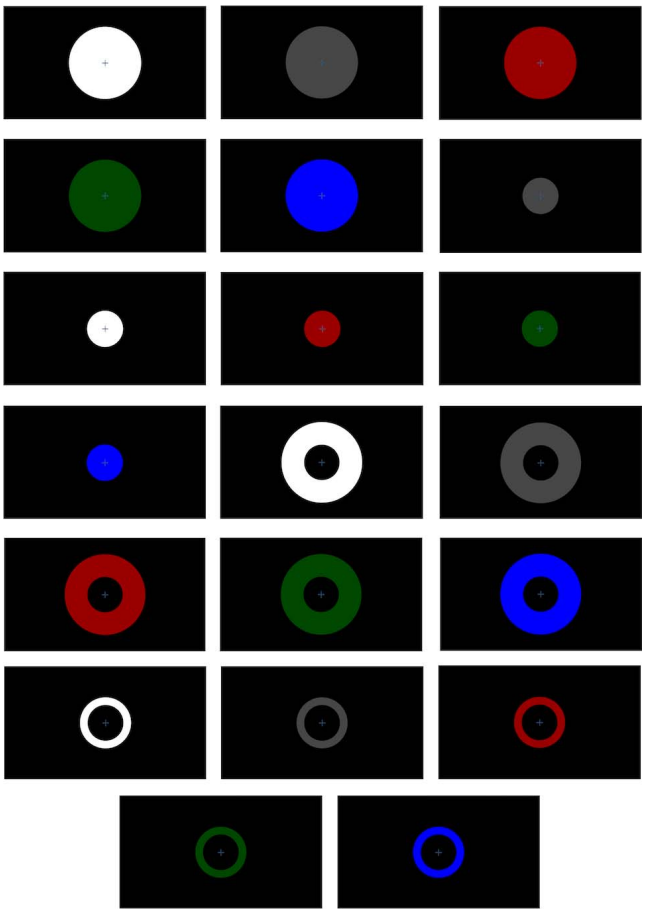

FIGURE 2. The 20 stimuli used to assess PA attributes in ACHM. All gray, red, green, and blue stimuli were designed to be isoluminescent (21 cd/m^2), while the white stimuli had a higher luminance (250 cd/m^2). From the *top left* are: the five large central circle stimuli (white, dim [gray], red, green, and blue); the five small central circle stimuli (vide supra); the five large peripheral annulus stimuli (vide supra); and the five small peripheral annulus stimuli (vide supra). All large stimuli (central circle or peripheral annulus) were designed to have an area four times that of the smaller stimuli (central circle or peripheral annulus).

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The illuminance of the large bright stimuli was 36.0 lux for both the peripheral and central stimuli, and for the large dim or colored stimuli was 7.0 lux for both the peripheral and central stimuli. These values for the small stimuli were 13.2 lux (bright) and 5.4 lux (dim or colored), respectively.

The large circle stimulus subtended a visual angle of 25.4°, and the small circle 12.7°. All of the peripheral (annulus) stimuli also had an inner diameter of 12.7°, with the outer diameters subtending 28.2° (large annulus) and 18.1° (small annulus). All large stimuli (central circle or peripheral annulus) had an area four times that of the smaller stimuli (central circle or peripheral annulus).

Before testing of any subject, and after the display monitor had been left on for 20 minutes, a luminance reading was taken of a standard calibration screen (the large white circle stimulus) to verify that the luminance was 250 cd/m^2, and of each of the dim, red, green, and blue stimuli to also verify that their luminance was 21 cd/m^2 as expected. The patient's head rested in a chin-rest supported on a height-adjustable table, maintaining the patient's eyes 40 cm from the monitor display screen.

Testing Strategy for Quantitative Subjective Measurement of PA Task. The patient was asked to look at a fixation target in the center of the screen at all times and the test was carried out binocularly. The patient was then presented with the background screen with fixation target and advised that three stimuli would now be presented (large white stimulus, followed by small gray stimulus, followed by small white stimulus) in order for the patient to familiarize themselves with the testing paradigm and the approximate range of stimulus brightness. The patient could rate these stimuli, although these ratings were not recorded. All stimuli throughout the experiment were presented for 2 seconds, with 5-second intervals between stimuli. The background screen then returned and the patient was instructed that recorded testing would begin. The 20 stimuli were subsequently presented in random order, each with a duration of 2 seconds before reverting to the black background screen, at which point the patient was asked to rate the stimulus on a subjective PA scale from 0 to 100, this score being recorded. Each patient completed three runs through the stimuli in succession, with a short break between runs.

Visual Acuity Measurements in Different Ambient Lighting Conditions

The best-corrected logMAR visual acuity (BCVA) of 26 ACHM patients was measured (in each eye and binocularly) in two ambient lighting conditions to assess the effect this had on recorded logMAR scores. We measured BCVA: (1) with standardized overhead fluorescent lighting on and the VA chart lightbox on. Precision Vision lightboxes (Precision Vision, Woodstock, IL, USA) were used, and were illuminated with two cool daylight 20-W fluorescent tubes (illuminance at patient viewing position of 400 lux and luminance 250 cd/m^2); and (2) with overhead lighting off and the VA chart lightbox on (illuminance at patient viewing position of 7 lux and luminance 155 cd/m^2).

Palpebral Aperture Measurements

The onset of palpebral aperture narrowing has been shown to correspond to the first reports of photophobic discomfort with increasing lighting conditions in normal subjects.[34] Given the striking finding of Zelinger et al.[22] in their binocular analysis of an approximately 50% reduction in palpebral aperture in achromats, we sought to explore this experimental paradigm in a larger number of patients to ascertain if these findings could be replicated and used as a further measure of PA in ACHM patients participating in clinical trials.

Palpebral Aperture Measurements – Methods. As per Zelinger et al.,[22] subjects were dark-adapted for 10 minutes while resting their head on a chin-rest in a custom-built Ganzfeld stimulator in complete darkness. After this period, standard video capture software was activated for a 10-second period, with input from an infrared camera located in the stimulator that recorded a video image of the subject's eye in the dark. After 5 seconds of video capture in the dark, the background light of the stimulator (which had been calibrated using a spectrophotometer to 0.6 cd m^{-2}) was activated and a further 5 seconds of video captured in the light ambient conditions. The vertical palpebral aperture height through the pupillary center in the dark and light ambient conditions was then measured using manual co-ordinate analysis of the eyelid positions on the captured video, using open source software to post-process the video segments (kinovea.org).

The vertical palpebral aperture in the dark was defined as 100%, and the corresponding percentage change in palpebral aperture with the onset of the light was then calculated using the co-ordinate analysis of the eyelid positions on the video image. A preliminary review of the first three achromat subjects tested suggested that the palpebral aperture narrowing we observed would not be nearly as substantial as the 50% reported by Zelinger et al.[22] when using 0.6 cd m^{-2}, and so an additional brighter light condition was introduced, following the same procedure as described above, also with a separate 10 minutes of dark adaptation preceding the palpebral aperture measurements and light onset. In the first instance, 70 cd/m^2 was tried, but the initial ACHM patients tested closed their eyes completely to this intensity and verbally reported that it caused them marked discomfort. A lower intensity of 16.6 cd/m^2 was subsequently tried, which was reported as bright but tolerable by the initial achromats tested.

Given that the initial treatment trials for ACHM have and are likely to administer therapy to one eye only, the relative effect of binocular versus uniocular PA would need to be borne in mind in a trial measure of PA. Given that binocular viewing, as assessed by Zelinger et al.[22], had been shown to be associated with a relatively lower photophobia threshold in migraineurs compared to uniocular viewing,[35] we also undertook monocular assessments of PA in our ACHM subjects, using the same conditions as described above, to ascertain if there was any difference between monocular and binocular viewing conditions.

Results

Demographics

A total of 26 molecularly proven ACHM patients completed the structured survey, quantitative subjective measurement of PA task, and VA measurements in different ambient lighting conditions task. Of these, 16 were male (62%) and 10 were female (38%), and their mean age was 32 years (SD, ±12.6 years). Of the patients in the study, 16 were *CNBG3* (61%); 4 were *CNGA3* (15%); 3 were *ATF6* (12%); 2 were *GNAT2* (8%); and 1 was *PDE6C* (4%).

Structured Patient Survey

All of the 26 ACHM patients (100%) surveyed indicated that light generally causes them discomfort. This was given a median rating of 70 out of 100 (on a scale of 0–100, with 0 being no discomfort and 100 being unbearable discomfort; IQR: 60–80). Interestingly, only 2 out of 26 (8%) patients said

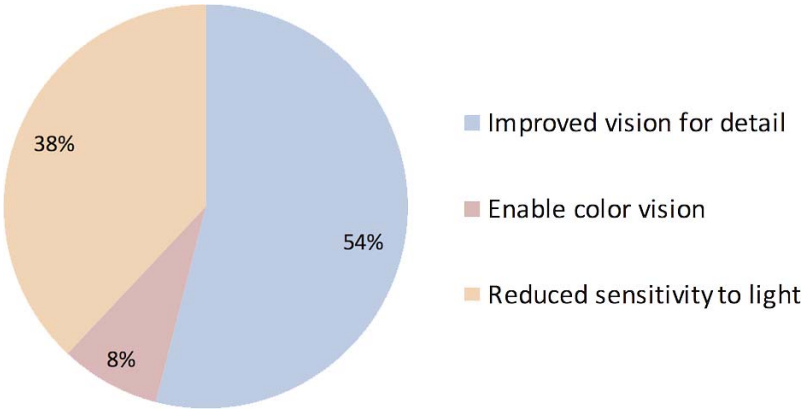

FIGURE 3. A pie chart showing the relative proportions of 26 ACHM patients that would choose to remedy one of the listed symptoms of ACHM above all others. It can be seen that a significant proportion (38%) would rate PA as the one aspect of their condition that they would most like to improve.

that bright lights caused them more issues with discomfort/pain as opposed to increased difficulty in seeing (24/26; 92%).

A total of 25 patients (96%) stated that they wore visual aids such as sunglasses to try and reduce PA. Of those that did, 8 (32%) wore them only outdoors; 1 (4%) wore them only indoors; and 16 (64%) wore them both indoors and outdoors. Also, 19 of these 25 patients (76%) reported that the need for such aids made them feel self-conscious or embarrassed in social situations.

Of those in the study, 23 of the 25 patients (92%) whose ADLs were affected by light sensitivity stated that this symptom limited their ability to carry out their ADLs; 18 (72%) said they had mild to moderate limitation in their ability to carry out ADLs; and 5 (20%) said they experienced marked limitation. One common theme among eight patients surveyed (31%) was that they felt their light sensitivity increased their perceived risk of accidents, and they stated that they had to take extra care to mitigate this, writing comments such as "increased risk factor of accidents" and limitation in "crossing roads."

The breakdown to question 10 *('Which one aspect of your vision would you improve?')* is show in Figure 3. A total of 14 (54%) patients said that they would choose improved vision for reading/visual acuity and only 2 (8%) said to enable color vision. Of particular interest, a significant proportion (10 patients [38%]) said that they would chose to improve their PA above all other aspects of their visual impairment.

Survey findings showed that 2 patients (6%) reported that PA has had no effect on their ability to do any kind of work; 16 (62%) indicated they had a mild to moderate limitation; and 8 (32%) said they had experienced a marked limitation in this regard. A total of 19 patients (73%) stated that they felt that their PA has impacted on their employment prospects, with 9 patients (35%) wrote comments about how their career choice was limited to not involving work outdoors.

Quantitative Subjective Measurement of PA

In this exploratory study, we used descriptive statistics to ascertain the effects of various stimulus attributes on subjective PA scores (SPAS). We aimed to answer several exploratory questions about PA in ACHM compared to normals, such as the effect of stimulus hue, intensity, location, and size. More complex models for analyzing the data were considered, but due to technical limitations preventing a fully factorial design and a limited sample size, we were not able to achieve suitable convergence for use of these statistical models.

The results of all testing conditions are shown in Figure 4. The score for each stimulus for each patient was the mean of three runs.

The most salient initial observation is that with either large or small stimuli, and with either central (circle) or peripheral (annulus) stimuli, the SPAS were significantly higher for the brighter (white stimuli (250 cd/m^2) as opposed to the dimmer (gray) or colored stimuli (all of which were isoluminescent at 21 cd/m^2) in both the normal and ACHM groups. Comparing the bright to dim stimuli alone, the mean SPAS scores were statistically significantly higher (paired *t*-test) for bright stimuli in all comparable conditions (i.e., size [large or small] and location [central circle or peripheral annulus]) in both normal and ACHM subjects (Table). This is reassuring evidence that this novel SPAS testing paradigm is broadly valid in terms of detecting greater subjective discomfort with more intense stimuli.

Given that the same pattern of results was seen across the four size/location combinations (as represented in the four graphs in Fig. 4), we further analyzed the data from the large central (circle) stimuli to examine the effect of color on SPAS between the four isoluminant stimuli (i.e., gray, red, green, and blue). We chose the large stimulus as this represented the higher illuminance (36.0 lux), and so would be expected to have the greatest effect on SPAS. In the normal group, there was a statistically significant difference between the SPAS scores across the different isoluminant colors ($P < 0.0001$; 1-way repeated measures ANOVA). The large red circle (LRC) SPAS (40 $\pm$ 15) and large blue circle (LBC) SPAS (41 $\pm$ 16) were both significantly higher than the isoluminant (gray) large dim circle (LDC) (16 $\pm$ 9); but in normals, they were not significantly different from each other ($P < 0.05$, Tukey's multiple comparison test). In the ACHM group, again there was a statistically significant difference between the SPAS scores across the different isoluminant colors ($P < 0.0001$; 1-way repeated measures ANOVA). However, unlike in the normal group, the ACHM group displayed a significant difference in their SPAS between the LRC and LBC stimuli, with the ACHM LRC SPAS (11 $\pm$ 12) being significantly lower than the ACHM LBC SPAS (37 $\pm$ 18; $P < 0.05$, Tukey's multiple comparison test; Fig. 5).

Again, taking the bright central (circle) stimuli for comparison, there were significantly higher SPAS for the larger stimuli compared to the smaller stimuli in both the normal (LWC SPAS = 62 $\pm$ 17 and small white circle (SWC) SPAS = 46 $\pm$ 17; $P < 0.0001$; paired *t*-test) and ACHM groups (LWC SPAS = 62 $\pm$ 20 and SWC SPAS = 47 $\pm$ 16; $P < 0.0001$; paired *t*-test).

There was little effect from the location of the stimuli (central versus peripheral). In the normal group the central

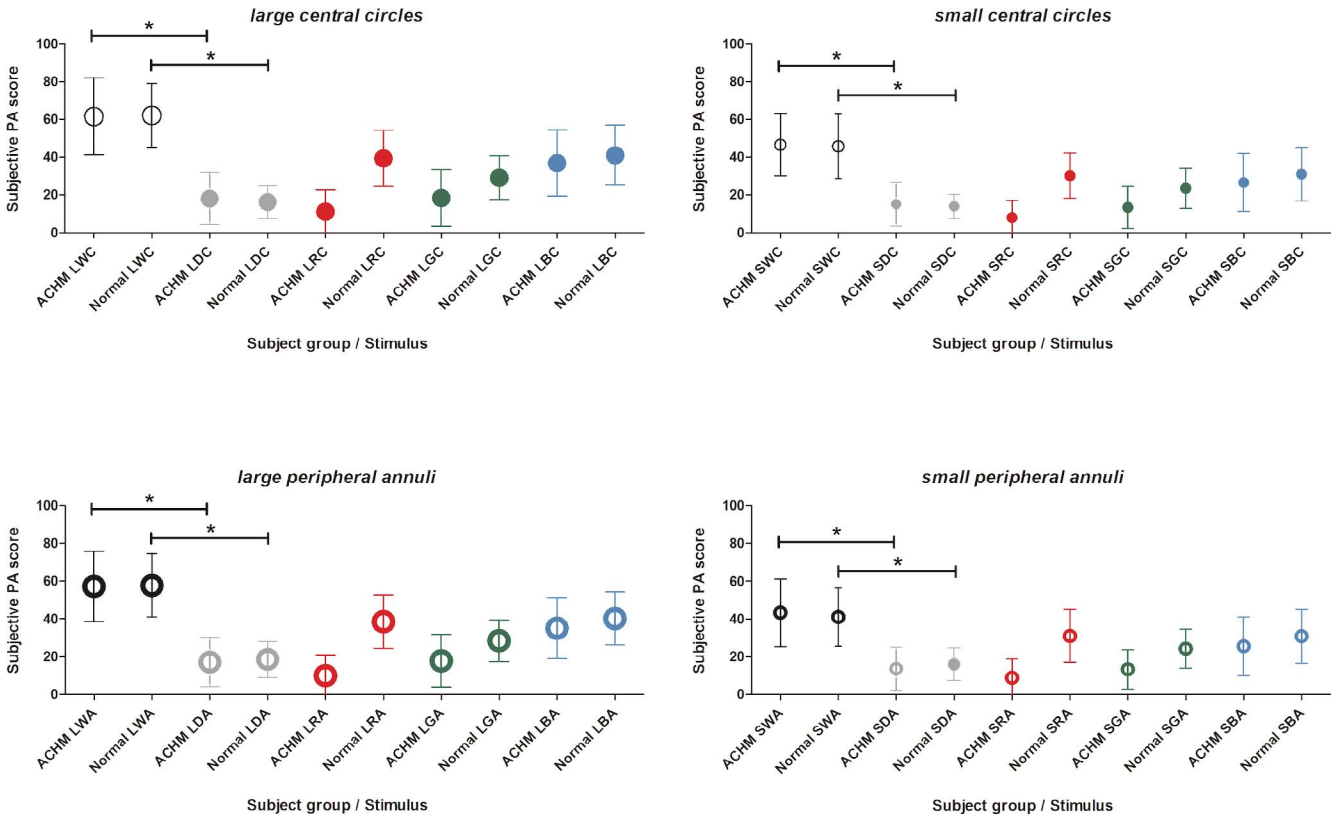

Figure 4. Photoaversion subjective quantification task results. The four graphs show the subjective PA scores of 26 ACHM and 26 normal subjects, scored out of 100 (see "Methods" section for definition). Each patient's score for each stimulus was the average of three runs. Shown are the mean (*symbols*) and SD (*vertical bars*) of each stimulus score (see stimuli descriptors below) for normals and ACHM subjects as indicated on the abscissa. Graph symbols are in the color and form of the stimulus they represent in order to aid ease of interpretation. Stimuli descriptors: L, large; S, small; W, white; D, dim (gray); R, red; G, green; B, blue; C, circle (central); A, annulus (peripheral; e.g., SDA, **s**mall **d**im **a**nnulus). * Indicates statistically significant difference between groups at $P < 0.0001$; paired *t*-test.

(LWC) SPAS = 62 ± 17 and the peripheral (large white annulus [LWA]) SPAS = 58 ± 17, and in the ACHM group, the central (LWC) SPAS = 61 ± 20 and the peripheral (LWA) SPAS = 57 ± 19; with the difference between location in each group being statistically insignificant ($P > 0.05$; paired *t*-test).

Visual Acuity Measurements in Different Ambient Lighting Conditions

Mean logMAR BCVA measurements were as follows in the two ambient lighting conditions tested: brighter ambient lighting conditions (250 cd/m^2): OD 0.89 logMAR (95% confidence interval [CI] 0.84–0.95); OS 0.95 logMAR (95% CI 0.88–1.0); and OU 0.88 logMAR (95% CI 0.83–0.94); dimmer ambient lighting conditions (155 cd/m^2): OD 0.89 logMAR (95% CI 0.84–0.95); OS 0.92 logMAR (95% CI 0.87–0.97); and OU 0.84 logMAR (95% CI 0.79–0.90).

There was no statistically significant difference between measured uniocular BCVA in the brighter and dimmer ambient lighting conditions in the right eye ($P = 0.98$; paired *t*-test) or the left eye ($P = 0.69$; paired *t*-test). However, there was a statistically significant difference between the mean BCVAs between conditions when measured binocularly ($P = 0.007$, paired *t*-test).

Table. Mean SPAS for ACHM and Normal Subjects Viewing Bright White Versus Dim Gray Stimuli (SD)

Normal Subjects				
LWC	62 (±17)	LDC	16 (±9)	$P < 0.0001$*
SWC	46 (±17)	SDC	14 (±6)	$P < 0.0001$*
LWA	58 (±17)	LDA	19 (±9)	$P < 0.0001$*
SWA	41 (±157)	SDA	16 (±8)	$P < 0.0001$*
ACHM Subjects				
LWC	62 (±20)	LDC	18 (±14)	$P < 0.0001$*
SWC	47 (±16)	SDC	15 (±13)	$P < 0.0001$*
LWA	58 (±19)	LDA	17 (±13)	$P < 0.0001$*
SWA	43 (±18)	SDA	14 (±11)	$P < 0.0001$*

LDA, large dim annulus; SWA, small white annulus.

* *P* values are as calculated by the paired *t*-test. Mean SPAS scores were significantly higher for bright stimuli in all comparable conditions (i.e., size [large or small] or location [central circle or peripheral annulus]) in both ACHM and normal subjects.

Results of Palpebral Aperture Measurements

A subset of 13 ACHM patients were tested with the lower light intensity used by Zelinger et al.[22] (0.6 cd/m^2) and of these, 10 also undertook the experiment with the brighter intensity (16.6 cd/m^2). We tested 10 normal subjects at both light intensities for comparison to the ACHM group.

The 13 ACHM patients who underwent palpebral aperture measurements (seven males [54%] and six females [46%]) had a mean age of 33 years (SD ± 12.01). Their genotype breakdown was six *CNGB3* (46%); three *CNGA3* (23%); two *ATF6* (15%); one *GNAT2* (8%); and one *PDE6C* (8%). The normals consisted of four males (40%) and six females (60%), with a mean age of 37 years (SD ± 11.82).

The mean percentage of dark-state palpebral aperture after a transition to a light of 0.6 cd/m^2 was 94% (SD ± 8%) in the

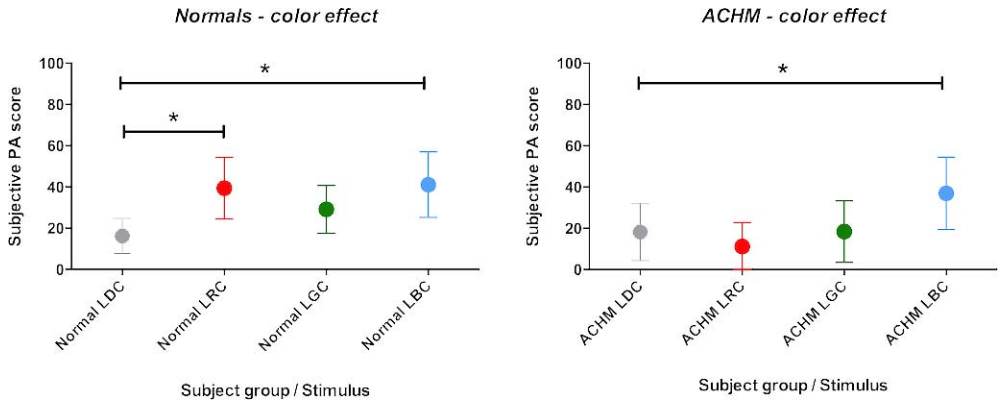

Figure 5. The color effect on SPAS in normals versus ACHM. All stimuli (*large central circles*) are isoluminant and their color is indicated by their stimuli indicator code (see text) on the abscissa and the data symbols' color. It can be seen that in the normal group, the blue and red stimuli score significantly higher SPAS than the dim (gray) stimuli despite being isoluminant; but in the ACHM group, only the blue stimuli score significantly higher SPAS than the dim (gray) stimuli. * Indicates statistically significant difference between groups at $P < 0.05$; Tukey's multiple comparison test.

ACHM group versus 98% (SD ± 6%) in the normal group. With the experiment repeated but with a transition to a brighter 16.6 cd/m^2, the mean percentage of dark-state palpebral aperture was 56% (SD ± 8%) in the ACHM group versus 92% (SD ± 10%) in the normal group (Fig. 6).

There was no statistically significant difference between the palpebral aperture narrowing in the ACHM versus normal group using the 0.6 cd/m^2 intensity light ($P = 0.21$; unpaired *t*-test), but with the 16.6 cd/m^2 intensity light the ACHM group had significantly more palpebral aperture narrowing that the normal group ($P < 0.0001$; unpaired *t*-test). These findings are reproducible when ACHM subjects are tested in monocular viewing conditions, and would be likely assessed in an interventional trial with treatment of one eye only (Fig. 6). Palpebral narrowing is not significantly different between binocular, monocular OD, and OS viewing conditions in the ACHM group ($P = 0.91$; Friedman test for the matched data points; and for the unmatched data points binocular versus OD eye $P = 0.75$, binocular vs. OS eye $P = 0.86$; Mann-Whitney *U* test).

Discussion

Structured Patient Survey

That 92% of patients in this study stated that bright lights caused them more problems with difficulty seeing, as opposed to discomfort/pain, indicates that the widely stated notion in the literature that ACHM patients suffer discomfort/photophobia with bright light may not represent the full picture of PA in ACHM. It may well be that visual image degradation with bright lights is also a significant issue for patients with ACHM. The observations of Aguilar et al.[32] that ACHM subjects had significantly ($P < 0.0001$) lower discomfort thresholds compared to normal subjects is interesting when viewed in the light of our results, where 92% of patients reported that reduced VA affects them more than discomfort. One might therefore infer from the combined results of this work and Aguilar et al.,[32] that both PA induced discomfort and hemeralopia are significant factors for patients in ACHM-associated PA. Two comments from the final question (Is there

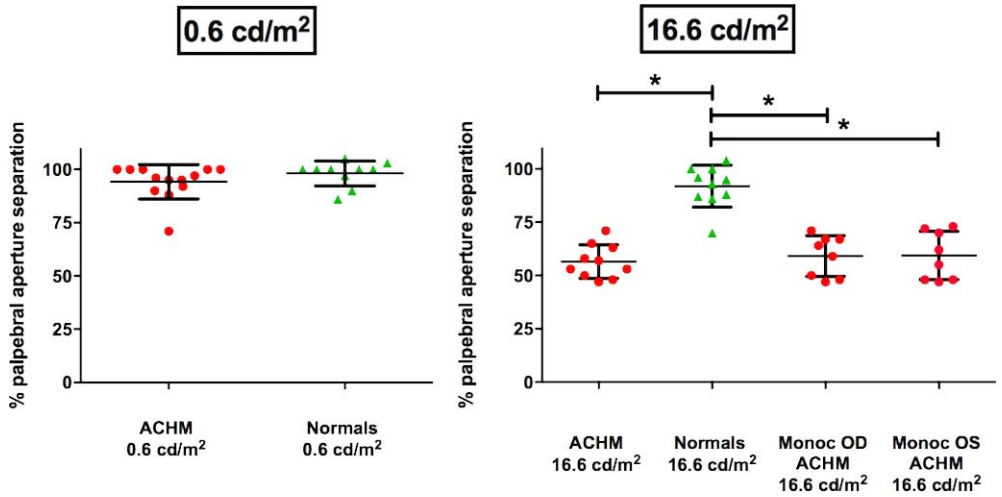

Figure 6. Plots of the relative palpebral aperture percentage after a transition from a dark state to one of two Ganzfeld stimulator lighting intensities (0.6 cd/m^2 and 16.6 cd/m^2 as indicated) in ACHM subjects (*red circles*) versus normal subjects (*green triangles*). *Horizontal plot lines* indicate the mean and *whiskers* indicate the SD of the values plotted. There is minimal difference in palpebral aperture narrowing between the ACHM and normal groups with the onset of a 0.6 cd/m^2 light from a dark state (binocular viewing; *left plot*); but with the onset of a 16.6 cd/m^2 light, the ACHM group has significantly more palpebral aperture narrowing than the normal group (binocular viewing; *right plot*). These findings are reproducible when ACHM subjects are tested in monocular right (OD) or left (OS) eye viewing conditions (*right plot*). * Indicates statistically significant difference between groups at $P < 0.0001$; 1-way ANOVA with Dunnett's multiple comparisons test.

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anything else you would like to add about light sensitivity that we have not covered?) appear to sum up, in the patients' own words, this message:

"It can be embarrassing having to wear sunglasses in even moderately bright places. It can be depressing at times also" and "basically all of my life is affected by light sensitivity in some form."

Our survey shows that PA is a major symptom in ACHM patients' lives, with 20% of patients experiencing marked limitation in their ability to carry out ADLs, and 38% saying that it is the one symptom that they would want to have remedied above all others (including VA [54%] or color vision [8%]). These data also demonstrate that measures such as sunglasses and tinted contact lenses may not fully alleviate all detrimental aspects of PA as experienced by patients. However, it must be kept in mind that given this was a survey about PA, this may have primed the subjects and introduced bias to their weighting of such a symptom.[36] Treatment of animal models has thus far been directed to restore cone function and visually directed behavior, but no assessment of PA has been undertaken.

We demonstrate that PA may also entail a socioeconomic burden, affecting ability to work (94%) and perceived employment prospects (73%), which suggest that there would also be socioeconomic benefits for ACHM patients in alleviating this symptom.

Quantitative Subjective Measurement of PA Task

That red and blue stimuli cause more photoaversion in normals than isoluminant gray stimuli had been previously reported,[24,25] and demonstrates that this testing paradigm is sensitive enough to corroborate previous observations. That the ACHM group has significantly lower SPAS for the red stimuli compared to either the blue or the dim (gray) stimuli is physiologically expected, given that the spectral sensitivity of the achromats' functioning rod photoreceptors (498 nm) is furthest away from the red stimuli. Whether the significantly higher SPAS scores for blue stimuli is due to rod input, a lack of cone input, ipRGC input, or a combination of these factors remains to be elucidated. The finding that larger stimuli also induced higher SPAS scores is also previously reported[30] and again a physiologically plausible effect. An interesting application of this paradigm would be to repeat the test in an ACHM group pre- and postintervention, and examine whether SPAS scores for red stimuli increased, to then mirror the normals' equivalence with blue stimuli. This would possibly indicate that there had been therapeutically induced LW-cone input to the PA sensation pathways.

The absolute SPAS ratings between ACHM and normals were very similar across all like-for-like comparisons. This might be explainable by the fact that this was a relative rating task, and so one might expect the psychometric scores to be similar given that on one hand ACHM patients are more photoaverse than normals (perhaps leading to higher SPAS scores than normals), but they also experience more PA in their daily lives, so the relatively low intensities used on the display monitor may induce relatively minor PA compared to the more severe PA that they normally endure (perhaps leading to lower SPAS scores than normals).

That there was little effect from the location of the stimuli (central versus peripheral) may be due to the fact that the peripheral stimuli were still relatively central. Stringham et al.[30] demonstrated that although PA thresholds increased (and therefore PA light sensitivity decreased) with retinal eccentricity, they did not increase significantly until at 20° eccentricity, whereas the large perimeter of the annulus stimuli used in this experiment only extended about 14° either side of fixation due to constraints in designing the stimuli size so they were consistent in area with the large central circle stimuli.

Visual Acuity Measurements in Different Ambient Lighting Conditions

In the two conditions tested, we found no statistically significant difference in uniocular BCVA between brighter or dimmer ambient lighting. However, we did find a statistically significant difference in binocular BCVA testing conditions. One possible explanation might be that this is a statistical but not clinically significant difference, given that the mean of the improved difference in the dimmer versus brighter ambient lighting condition in binocular assessments was small (0.04 logMAR, equivalent to only 2 letters on the ETDRS chart). However, given the results of the structured survey (where 92% of patients indicated that PA troubles them most through a reduction in detailed vision), it is likely that patients do experience some form of degradation in VA in brighter lighting conditions. It may be that the "bright" lighting conditions used herein were not bright enough; the illuminance of the "bright" ambient light setting was 400 lux (which might equate to the illuminance in an office or at sunrise/sunset) whereas illuminances might be considerably higher outdoors in other circumstances (e.g., 1000 lux on an overcast day or 10,000 lux in full daylight).

One other intriguing possible explanation for the difference in BCVA findings in uniocular versus binocular measurements may be related to summation of photophobia inputs between eyes. Wirtschafter et al.[35] demonstrated that the photophobia threshold in migraineurs is lowered (and therefore PA light sensitivity is increased) in binocular versus uniocular viewing. Therefore, an alternative explanation could be the larger effect of PA when viewed with both eyes. Further testing of patients in wider ranges of lighting conditions would be required to probe the extent of visual degradation and painful discomfort in ACHM with varying ambient lighting conditions. The consistency of ambient lighting conditions in recording ACHM patients' BCVA pre- and post-treatment should also be borne in mind.

Palpebral Aperture Measurements

Employing a light intensity of 16.6 cd/m^2 we observed a 56% eyelid separation in ACHMs and minimal change (92%) in the normal group. This contrasted with the results reported by Zelinger et al.[22] of 50% reduction in palpebral aperture in the three ACHM subjects that they tested using a lower light intensity of 0.6 cd/m^2. The reason for this difference is not clear. Our larger ACHM group included various genotypes, whereas their group only included *CNGA3* subjects. A post-hoc subgroup analysis of our data revealed that the mean palpebral aperture using 0.6 cd/m^2 in the *CNGA3* subgroup was 98% (SD $\pm$ 3%) versus 90% (SD $\pm$ 11%) in the other genotypes combined; and using the 16.6 cd/m^2 light, the *CNGA3* subgroup was 58% (SD $\pm$ 12%) versus 56% (SD $\pm$ 7%) in the other genotypes combined. It appears that the *CNGA3* subgroup is not significantly more or less photoaverse than other genotypes combined, although the relatively low number of patients of each genotype tested precludes more statistical cross-group comparisons. It would be of interest to carry out these experiments in larger numbers of each genotype to determine if there is any difference between genotypes.

Notwithstanding the above, this experiment suggests that an intensity of 16.6 cd/m^2 would be an appropriate luminance setting to assess PA-induced palpebral aperture changes in ACHM pre- and post-treatment, given the statistically significant difference in palpebral aperture narrowing in ACHM patients

using this luminance versus no significant palpebral aperture narrowing in the normal group. Importantly, we have also established that this light level produces similar results in the monocular viewing condition when compared to the binocular viewing condition; this is relevant as any therapeutic trial is likely to treat only one eye.

In conclusion, this study demonstrates—with an evidence base for the first time in the literature—that PA is a significant symptom for ACHM patients. This may have implications for the direction of development of future treatment strategies for ACHM. The evidence from this study suggests that, given the importance of this symptom to many ACHM patients, PA could be considered as an additional outcome measure in therapeutic trials. We have also shown for the first time that PA in ACHM is due more to the perceived visual degradation of the patient's visual image, as opposed to a physical sensation of pain or discomfort. This has implications for doctors' understanding of what ACHM patients actually experience, and suggests that the classical term "photophobia," which is used ubiquitously in the ACHM literature, should be replaced with the term "photoaversion," since the former is commonly held to mean a noxious sensation induced by normal light levels.

We have developed a quantitative SPAS task that can be used to assess ACHM patients' PA without subjecting them to the bright light intensities that have been used in traditional threshold photophobia testing paradigms at use in other conditions, and have developed a normative group dataset in this task for comparison. We have further developed an objective measure of PA in the existing palpebral aperture testing method that reliably distinguishes normals from ACHM in monocular viewing conditions most likely to be relevant to future treatment interventions, and have shown that the light intensities previously published in a very small number of ACHM patients using a similar method are unlikely to be intense enough, and have suggested a suitable light intensity. This SPAS testing paradigm and the palpebral aperture settings, in addition to the structured survey, may be useful in monitoring the significant symptom of PA pre-and post-interventional gene therapy trials, with hopeful improvement of these parameters following treatment.

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