Pupillary Light Reflex Reveals Melanopsin System Alteration in the Background of Myopia-26, the Female Limited Form of Early-Onset High Myopia

Mirella T. S. Barboni,[1] Noémi Széll,[2] Zoltán Sohajda,[3] and Tamás Fehér[4]

[1]Department of Ophthalmology, Semmelweis University, Budapest, Hungary
[2]Department of Ophthalmology, University of Debrecen, Debrecen, Hungary
[3]Kenézy Campus Department of Ophthalmology, University of Debrecen, Debrecen, Hungary
[4]Institute of Biochemistry, Biological Research Centre, Szeged, Hungary

Correspondence: Mirella T.S. Barboni, Department of Ophthalmology, Semmelweis University, Mária u. 39, Budapest 1085, Hungary; mtsbarboni@gmail.com.

MTSB and NS contributed equally to this study.

Received: September 19, 2023
Accepted: May 17, 2024
Published: July 3, 2024

Citation: Barboni MTS, Széll N, Sohajda Z, Fehér T. Pupillary light reflex reveals melanopsin system alteration in the background of myopia-26, the female limited form of early-onset high myopia. *Invest Ophthalmol Vis Sci.* 2024;65(8):6. https://doi.org/10.1167/iovs.65.8.6

Purpose. The purpose of this study was to evaluate pupillary light reflex (PLR) to chromatic flashes in patients with early-onset high-myopia (eoHM) without (myopic controls = M-CTRL) and with (female-limited myopia-26 = MYP-26) genetic mutations in the *ARR3* gene encoding the cone arrestin.

Methods. Participants were 26 female subjects divided into 3 groups: emmetropic controls (E-CTRL, $N = 12$, mean age = 28.6 ± 7.8 years) and 2 myopic (M-CTRL, $N = 7$, mean age = 25.7 ± 11.5 years and MYP-26, $N = 7$, mean age = 28.3 ± 15.4 years) groups. In addition, one hemizygous carrier and one control male subject were examined. Direct PLRs were recorded after 10-minute dark adaptation. Stimuli were 1-second red (peak wavelength = 621 nm) and blue (peak wavelength = 470 nm) flashes at photopic luminance of 250 cd/m^2. A 2-minute interval between the flashes was introduced. Baseline pupil diameter (BPD), peak pupil constriction (PPC), and postillumination pupillary response (PIPR) were extracted from the PLR. Group comparisons were performed with ANOVAs.

Results. Dark-adapted BPD was comparable among the groups, whereas PPC to the red light was slightly reduced in patients with myopia ($P = 0.02$). PIPR at 6 seconds elicited by the blue flash was significantly weaker ($P < 0.01$) in female patients with MYP-26, whereas it was normal in the M-CTRL group and the asymptomatic male carrier.

Conclusions. L/M-cone abnormalities due to *ARR3* gene mutation is currently claimed to underlie the pathological eye growth in MYP-26. Our results suggest that malfunction of the melanopsin system of intrinsically photosensitive retinal ganglion cells (ipRGCs) is specific to patients with symptomatic MYP-26, and may therefore play an additional role in the pathological eye growth of MYP-26.

Keywords: early-onset high-myopia (eoHM), melanopsin, intrinsically photosensitive retinal ganglion cells (ipRGCs), pupillary light reflex (PLR), cone-arrestin, *ARR3*, Myopia-26 (MYP-26)

Early onset high myopia (eoHM) is often the result of a single gene mutation which, in turn, displays a Mendelian form of inheritance. Among the genes most often involved in Mendelian eoHM, is the X-linked *ARR3*, encoding the cone arrestin. The resulting disease, sometimes referred to as Myopia-26 (MYP-26; MIM: 301010), was first reported in an Asian cohort[1] and was more recently documented in Caucasian families.[2,3] Counterintuitively, MYP-26 is a female-limited disease: heterozygous female patients display eoHM that progresses to a degenerative stage with aging, whereas hemizygous male patients for the mutant allele retain good vision. This disease is associated with myopic fundus alterations caused by the pathological eye elongation, but no abnormalities typical of cone dystrophy are present. Low visual acuity is found in female patients but not in male patients,[3–6] as in the cone arrestin null mouse model,[7] whereas color vision deficiency is present in both hemizygous male and heterozygous female patients.[3,4] Importantly, the cone dysfunction indicated by the above symptoms is not a consequence of the eye elongation but seem to be directly caused by the *ARR3* gene mutations.[4] It may play a role in the pathological mechanism leading to high myopia (HM), but the gender-specific nature of this connection is still under investigation.[4]

In cones, ARR3 or cone arrestin (MIM: 301770), the product of the *ARR3* gene, binds the light-activated phosphorylated receptors to shutoff opsin signaling during the phototransduction cascade. Therefore, its alteration affects the photoreceptor deactivation.[8,9] Gu et al. recently reported that patients with MYP-26 may show normal S-cone and abnormal L/M cone function, probably due to compensatory mechanisms of the rod arrestin expressed in S-cones.[4] The authors raised a hypothesis to explain the female-limited profile of HM in MYP-26. They stated that the random X-chromosome inactivation causing a heterogeneous pattern of L/M-cone dysfunction may induce pathological eye growth due to the resulting constitutive contrast signal in the retina, whereas L/M-cones homogeneously affected in hemizygous male patients would not.[4]

In addition to its role in the phototransduction cascade, cone arrestin interacts with D2-like dopamine receptors, mainly the D4 receptor (DRD4), highly expressed in photoreceptors.[10] DRD4 is involved in the circadian control of rod/cone gap junction coupling[11,12] and in the light-dependent intrinsic dopamine-melatonin retinal circadian clocks which, in turn, influence and are influenced by the function of melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) in the vertebrate retina.[11,13] The ipRGC-melanopsin system disruption by either genetic[14,15] or environmental factors (i.e. light)[16–18] has been consistently reported to alter refractive development toward a myopic shift. The current assumption is that the melanopsin-driven endogenous circadian clock in the retina plays a crucial role in emmetropization.[19] The potential influence of the melanopsin system on myopic development is also supported by the association between the prevalence of myopia and the time spent outdoors under natural sun light.[20,21] However, to establish a more complete picture on the influence of the time spent outdoors on myopic development, the circadian regulation of gene expression needs to be considered. Recently, a meta-analysis of a large population from European ancestry revealed that genetic factors controlling circadian rhythm are involved in the development of myopia and refractive errors.[22] Overall, *ARR3* gene mutations could prove to be an extreme example of genetic factors influencing ipRGC-melanopsin function, and thereby emmetropization.

The assessment of ipRGC-melanopsin system integrity is feasible through the ipRGCs controlling the pupillary light reflex (PLR), by investigating the postillumination pupillary response (PIPR) specifically originating in the ipRGCs.[23–27] Whereas photoreceptors (cones and rods) sending inputs to ganglion cells are responsible for the pupillary constriction, melanopsin expressed in ipRGCs is the main generator of PIPR[24] under a spectral environment (short-wavelength) providing the optimal condition for melanopsin system activation in primates.[28]

In this study, we recorded PLRs from female patients with MYP-26 with *ARR3* gene mutations, from an asymptomatic male carrier, from patients with other forms of eoHM, and from emmetropic controls.

METHODS

The procedures were performed in accordance with the Declaration of Helsinki (1964) and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants included in the study, or their legal representatives. The protocols and procedures have been approved by the National Scientific and Research Ethics Committee of the Medical Research Council of Hungary (registration number 58542-1/2017/EKU).

Participants

The clinical and demographic characteristics of the patients with eoHM enrolled in this study are summarized in Table 1. Early onset was defined as myopia initiation between 2 and 3 years of age, based on patients' anamnesis. HM was declared as spherical equivalent (SE) ≤ -6.0 Diopters (D). Participants were 14 female patients with eoHM (see below), 12 female healthy volunteers, 1 male carrier, and 1 male control. All eoHM subjects underwent ophthalmological and genetic examinations partially reported in Széll et al. (2021).[3] Refractive error was determined by non-cycloplegic autorefraction using an automated kerato-refractometer (except for patients under 18 years of age measured under cycloplegia). Best-corrected visual acuity (BCVA) was measured using trial lenses with the correction providing the best visual acuity.

Seven female patients (mean age = 28.3 ± 15.6 years, mean SE = −10.25 ± 3.22 D), all related to each other (see the pedigree in Fig. 1) displayed *ARR3* heterozygous mutant genotype (the MYP-26 group), with one of the alleles harboring an early nonsense mutation that completely inactivates the encoded protein (c.214C>T, p.Arg72*).[3] The other seven female patients (mean age = 25.7 ± 11.5 years, mean SE = −9.55 ± 2.46 D) showed *ARR3* homozygous wild type genotype (the myopic control [M-CTRL] group). Emmetropic controls (E-CTRLs) were age-matched (mean age = 28.6 ± 7.8 years) healthy female volunteers with negligible refractive errors (SE = −0.50 to +0.50 D), normal BCVA in both eyes, and absence of ocular or systemic diseases that could affect the central nervous system. In addition, results from 2 male subjects were included: one asymptomatic male subject member of the family of the inter-related ARR3 heterozygous

TABLE 1. Characteristics of Patients Included in This Study

	Female Groups			Male Subjects	
	E-CTRL	M-CTRL	MYP-26	Male Carrier	Male Control
N (eyes)	12 (24)	7 (14)	7 (14)	1 (2)	1 (2)
Age, y	28.6 ± 7.8	25.7 ± 11.5	28.3 ± 15.6	37	37
SE, diopters	−0.50 to +0.50	−9.55 ± 2.46	−10.25 ± 3.22	−1.00 and −2.00	−2.50 and −2.50
BCVA, decimal	1.00	1.00	0.41 ± 0.25	1.00 and 0.70[*]	1.00 and 1.00
Fundus[†]	C0	C1	C1/C2	C0	C0
Macular OCT	Not measured	Normal retinal and choroidal structure	Thinned choroid visible sclera[‡]	Normal retinal and choroidal structure	Not measured

Quantitative data of female groups are depicted as means ± standard deviations (SD).

[*] Strabismic amblyopia caused by exotropia.

[†] Fundus appearance according to META-PM.[29]

[‡] The optical coherence tomography (OCT): intact photoreceptor layer in all patients with MYP-26.

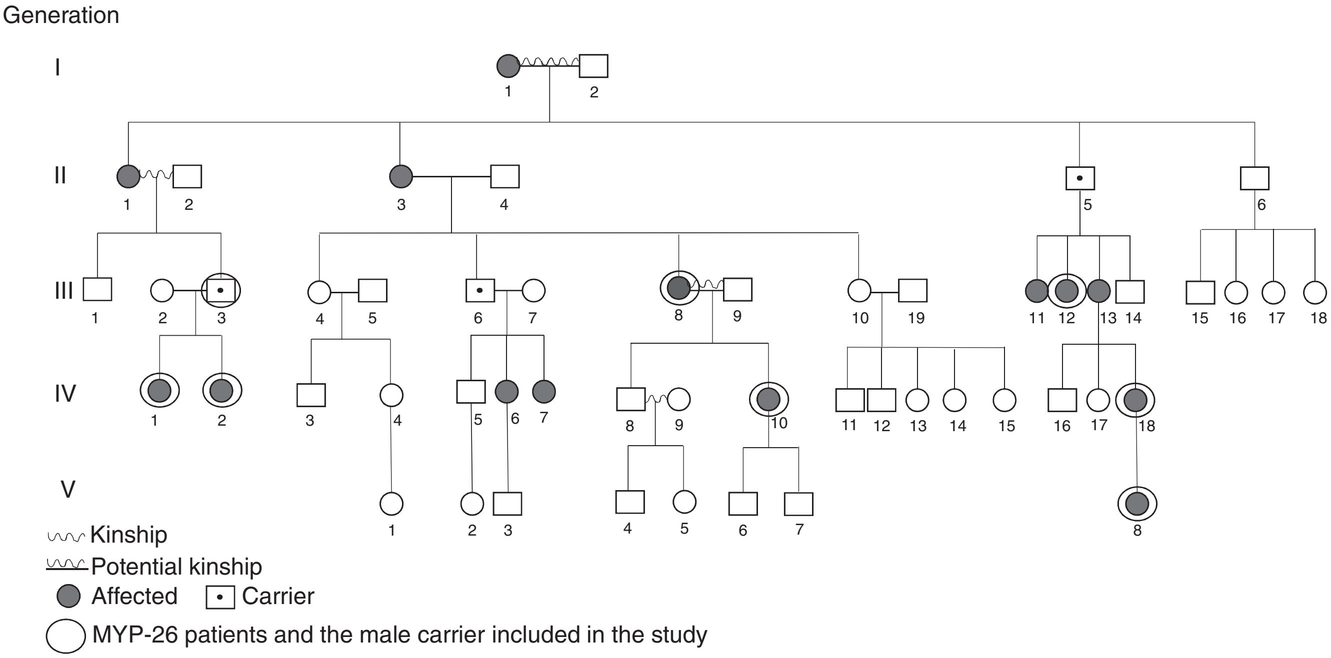

Figure 1. Pedigree of the family with X-linked female-limited eoHM (Myopia-26).

women (37 years old, SE = −1.00/−2.00 D, subject III/3 in the pedigree) with confirmed hemizygous *ARR3* gene mutation, and one age-matched control subject.

Two-Color Pupillary Light Reflex

In this study, we used the handheld RETeval portable system to record the two-color PLR, as recently reported by Asakawa et al.[30] This was a single session/single trial measurement, except for the male carrier who underwent PLR recordings twice in the same session. Figure 2A shows that the PLR protocol started with 10-minute dark-adaptation to allow the largest possible and stable baseline pupil diameter (BPD) to be recorded prior to light stimulation. After dark-adaptation, PLR to the red short-flash was recorded on the right eye, whereas the left eye was kept completely occluded. After 2 minutes of the dark-adaptation interval, the same measurement was performed on the left eye, while the right eye was covered. After another 2-minute dark-adapted interval, the same procedure was repeated for the blue short-flash stimulation. A dark-adaptation interval of 2 minutes was again applied between the measurements of the 2 eyes. If a large artifact or a measurement error was detected by the examiner, that condition was retested after a 2-minute dark-adaptation period.

The integrated infrared camera provided 0.08 mm/pixel resolution at the iris and 28.3 hertz (Hz) recording rate. Visual stimuli were 1-second flashes of light. The photopic luminance was 250 cd/m^2 in both red (relative spectral energy between 590 and 650 nm; peak wavelength = 621 nm) and blue (relative spectral energy between 430 and 510 nm; peak wavelength = 470 nm) light stimuli. It has been well established that long-wavelengths fall away from the peak of the melanopsin absorption spectrum. Therefore, PLR to the red light contain insignificant direct activation of the ipRGC-melanopsin system. On the other hand, the short wavelengths are able to activate melanopsin directly,[24,31] besides activating the outer retinal photoreceptors. Figure 2B shows that, accordingly, PLR to red (long-wavelength) flash shows a fast pupil constriction and redilation, which is driven by cone inputs to ipRGCs, whereas PLR to the blue (short-wavelength) flash shows rod/cone controlled fast pupil constriction, followed by the slow PIPR driven by the ipRGC-melanopsin system.[23]

Data Analysis

PLRs to the red and blue flashes of light were analyzed with MatLab (MathWorks Inc., Natick, MA, USA) custom-written program. As shown in Figures 2B and 2C, the BPD was calculated for both responses elicited by the red and blue flashes. The BPD was the average pupil diameter during the 1-second recording preceding each stimulus onset. The peak pupil constriction (PPC) was calculated as the difference in normalized pupil diameters between BPD and maximal constriction after flash offset. The PIPR was calculated twice for each condition (red and blue): at 6 seconds (PIPR6) and at 30 seconds (PIPR30) after flash offset. PIPR6 and PIPR30 indicated the differences between BPD and pupil diameters measured 6 and 30 seconds after the flash, respectively, normalized to the BPD. PPC as well as PIPR were normalized to correct for individual differences in BPD, as previously recommended.[25,32] Statistical comparisons were applied to pooled right and left eyes for the female groups. Genotype and group comparisons were performed using 1 or 2-way ANOVAs depending on the presence of a within-subject repeated measure. The P values < 0.05 were considered statistically significant. Special attention was paid to the potentially lower variation of the data deriving from the MYP-26 group (comprising related individuals only), as it might lead to easier rejection of the null-hypothesis of statistical tests, and consequently an increased rate of false positive statistical comparisons. For no parameter, however, did the MYP-26 group display the lowest SD

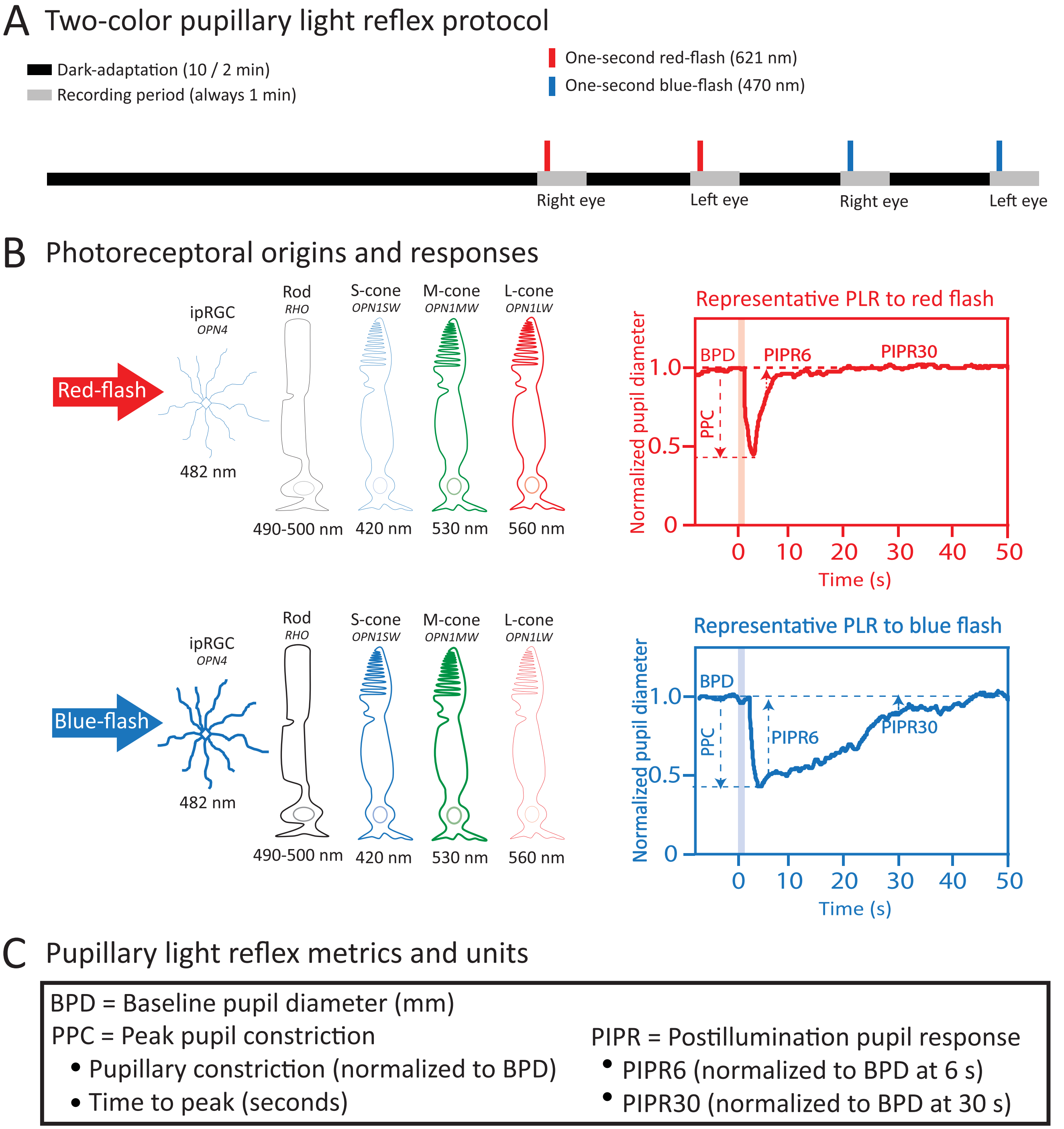

Figure 2. Pupillary light reflex (PLR) protocol, origins, responses, and metrics. (**A**) The PLR protocol applied alternating dark-adaptation (*black*) and recording (*grey*) periods. The latter lasted 1 minute each, and comprised a 1 second red or blue light flash, delivered 10 seconds after the start of recording. (**B**) *Left*: The five types of human retinal photoreceptors are differentially activated depending on the flash stimulus: the red flash mainly activates the L/M cones (*darker drawing*), whereas the blue flash activates ipRGCs directly and the outer retinal photoreceptors with the L-cones being less activated (*lighter drawing*). *Right*: From a representative emmetropic control subject of the study, the PLR to the red flash (*upper plot*) elicited fast pupil constriction and redilation with weak postillumination pupil response (PIPR) at 6 and 30 seconds after stimulus offset. On the other hand, the PLR to the blue flash generated a sustained pupil constriction, that is, a strong PIPR after the peak pupil constriction (PPC) and before recovery to the baseline. (**C**) PLR metrics and units.

Table 2. The Results of PLR Measurements

	Female Groups			Hemizygous Male Carrier		
PLR	**E-CTRL**	**M-CTRL**	**MYP-26**	**Test 1**	**Test 2**	**Age-Matched Male-Control**
Red Flash						
BPD	6.21 ± 0.57	6.06 ± 0.79	5.91 ± 1.36*	6.59	6.55	5.93
PPC	0.54 ± 0.05	0.47 ± 0.07	0.47 ± 0.06	0.49	0.44	0.56
PIPR6	0.14 ± 0.08	0.12 ± 0.06	0.13 ± 0.06	0.19	0.17	0.19
PIPR30	−0.01 ± 0.05	−0.02 ± 0.09	−0.02 ± 0.05	0.05	0.02	−0.04
Blue flash						
BPD	6.03 ± 0.72	6.16 ± 0.82	5.87 ± 1.36*	6.29	6.37	5.95
PPC	0.60 ± 0.05	0.58 ± 0.03	0.57 ± 0.04	0.63	0.62	0.60
PIPR6	0.51 ± 0.06	0.50 ± 0.05	0.42 ± 0.07	0.55	0.55	0.51
PIPR30	0.13 ± 0.12	0.11 ± 0.05	0.12 ± 0.09	0.24	0.18	0.13

Mean + SD is shown for female groups.

* All patients with MYP-26, including the outlier patient (outlier data: BPD1: OD = 3.2; OS = 3.6; and BPD2: OD = 3.7; OS = 3.1 mm).

BPD = pupil diameter (mm). PPC and PIPRs = pupil constriction (difference between BPD and pupil diameters, normalized to the BPD, as shown in Fig. 2B).

among the 3 groups analyzed (see Tables 1, 2), indicating that this potential source of error was unlikely to mislead the study.

RESULTS

Ophthalmological examinations revealed normal anterior and posterior segments of the eye, except for the myopic fundus alterations, high myopic refractive error of SE −5.50 to −16.00 D, and reduced BCVA for the patients with MYP-26. The M-CTRLs also showed normal anterior and posterior segments and HM between SE −6.00 and −14.00 D, but all these patients achieved full BCVA = 1.0 (decimal). Statistical comparisons showed similar mean age among the three groups ($F_{(2,25)} = 0.157$; $P = 0.856$) as well as comparable SE between groups MYP-26 and M-CTRL ($P = 0.999$). However, BCVA was significantly reduced in patients with MYP-26 as compared to the M-CTRL and E-CTRL groups ($F_{(2,51)} = 115.990$; $P < 0.001$). All subjects in the E-CTRL group showed no relevant refractive error (SE = −0.50 to +0.50 D) and had normal ophthalmological findings. The male subjects (carrier and healthy control) had normal anterior and posterior segments, low myopic refractive errors, and full BCVA for the age-matched male control and the dominant eye of the male carrier. The non-dominant eye of the male carrier showed reduced BCVA due to exotropic amblyopia. No other ocular diseases were observed in any of the patients.

The fundus appearance and the optical coherence tomography (OCT) images of patients with MYP-26 showed no characteristics of cone dystrophy, such as bull's eye, macular geographic atrophic lesions, or outer retinal changes (i.e. disruption and loss of the photoreceptor layer), as opposed to what was expected based on the ARR3 knockout animal model.[7] Retinal morphology was rather characteristic of HM and comparable in both the M-CTRL and MYP-26 groups. The Meta-Analyses for Pathologic Myopia (META-PM) classification system was used to categorize myopic retinal changes.[29] Category 0 (C0 = normal retinal structure) was observed in the E-CTRL and in the male carrier. Category 1 (C1 = tessellated retina) was observed in all M-CTRL subjects, whereas category 1 or 2 (C2 = diffuse chorioretinal and peripapillary atrophy) was observed in patients with MYP-26. OCT images revealed normal retinal and choroidal structure for the M-CTRL and male carrier patients, whereas moderate to extreme attenuation of the choroidal thickness without disruption or loss of the photoreceptor layer corresponding to C2 fundus appearance for the patients with MYP-26. Advanced stages of pathological fundus alterations (META-PM C3 and C4), signs of posterior staphyloma or visual media opacity (cataract, corneal, or vitreous opacities), that could affect PLR were not observed.

Baseline Pupil Diameter

Prior to the flash onset, pupil size was recorded for 9 seconds. The BPD was the average of the pupil diameter recorded during the last second before the flash onset for the red condition BPD1 and for the blue condition BPD2. First, we found that BPD was highly replicable based on the coefficient of variance (CoV) which was less than 5% in the 3 groups (mean CoV: E-CTRL = 4.5%, M-CTRL = 2.7%, and MYP-26 = 3.4%) considering the two BPD measurements. The 2-way ANOVA within-subject repeated measure (BPD1/BPD2) was applied to compare the mean BPDs among the 3 groups. The mean BPDs are shown in Table 2. BPDs were statistically comparable among the groups, irrespective of considering one outlier subject from the MYP-26 group with relatively small BPDs (outlier included: group effect: $F_{(2,49)} = 0.313$ and $P = 0.733$; BPD*condition interaction: $F_{(2,49)} = 2.016$ and $P = 0.144$), or not (outlier excluded: group effect: $F_{(2,47)} = 0.296$ and $P = 0.745$ and BPD*condition interaction: $F_{(2,47)} = 2.060$ and $P = 0.139$). When searching for potential associations between BPD and age, we found significant negative correlations (BPD1 × age: $r = -0.608$, $P = 0.021$ and BPD2 × age: $r = -0.611$, $P = 0.020$; Supplementary Fig. S1). It is worth mentioning that BPDs recorded after dark-adaptation may not be comparable to pupil sizes of patients with myopia reported by previous studies due to differences in light conditions.

Peak Pupil Constriction

Figure 3 shows PPC amplitude and time to peak constriction comparisons among the three groups for the red (upper

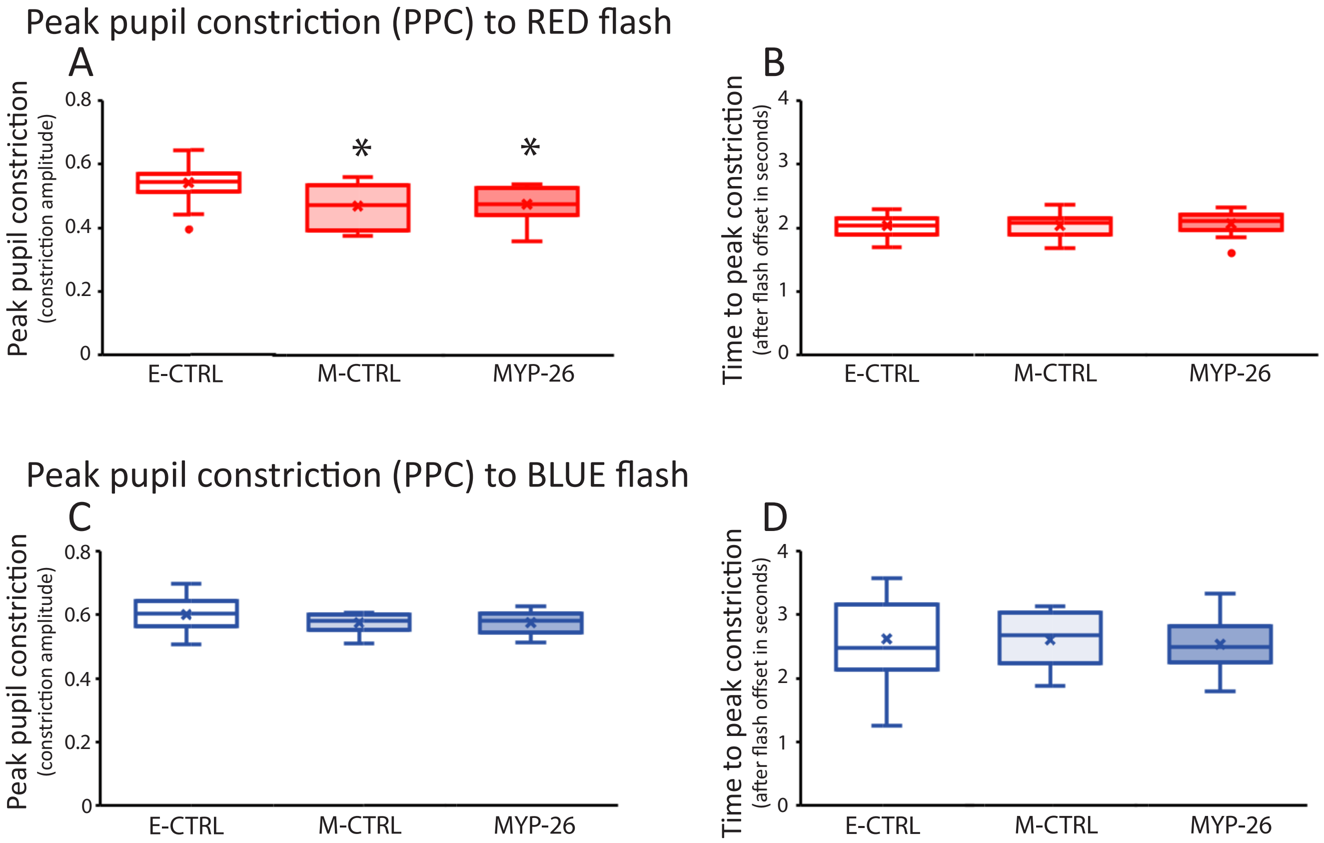

Figure 3. **Peak pupil constriction (PPC) normalized to baseline** for E-CTRL (empty symbols), M-CTRL (light color), MYP-26 (dark color). (**A**) Mean PPCs to the red flashes were statically different among the groups with patients with myopia showing decreased values (less constriction, marked with *asterisks*) compared to the emmetropic control group. (**B**) Comparable time to peak (approximately 2 seconds for the 3 groups) was observed for the PPC to the red flash. (**C**) The PPC to the blue flash was statistically similar among the groups. (**D**) Time to peak constriction to the blue flash was highly variable, as expected. No statistical differences were observed among the groups for the time to peak constriction to the blue flash. * Significant ($P < 0.05$) values with post hoc Bonferroni test when compared to control group (E-CTRL).

plots) and the blue (bottom plots) conditions. The PPC to the red flash was reduced in patients with myopia. The mean PPCs are shown in Figure 3A and Table 2. One-way ANOVA showed that PPC was significantly lower in myopes (group effect: $F_{(2,49)} = 8.780$; $P = 0.001$). Bonferroni post hoc (between groups) comparisons revealed a myopic effect on PPC to the red flash because both myopic groups were similarly reduced (M-CTRL versus MYP-26: $P = 0.999$) compared to E-CTRL ($P = 0.02$). Time to peak constriction after the red flash offset (Fig. 3B) was comparable between the myopic groups (mean M-CTRL = 2.04 ± 0.19 seconds and MYP-26 = 2.07 ± 0.20 seconds) and they were both similar to the control values (mean E-CTRL = 2.03 ± 0.16 seconds). Significant differences among the groups were not observed for the time to peak constriction to the red flash (group effect: $F_{(2,49)} = 0.244$; $p = 0.784$). Overall, myopia seems to influence PPC to the red flash in our cohort, as shown in detail in Figure 4A.

PPC to the blue flash was comparable (group effect: $F_{(2,49)} = 2.206$ and $P = 0.121$) among the groups with very similar means (see Fig. 3C, Table 2). Moreover, the mean time to peak constriction (Fig. 3D) was similar among the groups (E-CTRL = 2.63 ± 0.65, M-CTRL = 2.61 ± 0.46, and MYP-26 = 2.54 ± 0.39), and more variable compared to the time to peak of the PPC to the red flash, as expected. The statistical comparisons revealed no significant differences (group effect: $F_{(2,49)} = 0.129$ and $P = 0.879$) among the control and myopic groups for the time to peak.

Postillumination Pupillary Response

Figure 5 shows PIPR to the red (upper plots) and the blue (bottom plots) conditions at 6 seconds (PIPR6) and at 30 seconds (PIPR30) after flash offset. PIPR6 values are higher in the blue condition compared to the red condition, as expected (see Table 2). Group comparisons showed that PIPRs to the red flash were similar for both PIPR6 (Fig. 5A) and PIPR30 (Fig. 5B) among the groups (PRPR6 group effect: $F_{(2,49)} = 0.390$ and $P = 0.679$ and PIPR30 group effect: $F_{(2,49)} = 0.265$ and $P = 0.768$).

In contrast, significantly reduced PIPR6 was found in the blue flash condition for the MYP-26 group (Fig. 4B shows the average PLR traces and PIPR in detail). The mean E-CTRL PIPR6 to the blue flash (Fig. 5C) was similar to the mean of the M-CTRL group. However, the MYP-26 group showed significantly lower PIPR6 compared to both control groups E-CTRL and M-CTRL. The 2-way ANOVA reflected these differences (group effect: $F_{(2,49)} = 10.392$ and $P < 0.001$), whereas the post hoc Bonferroni test showed that

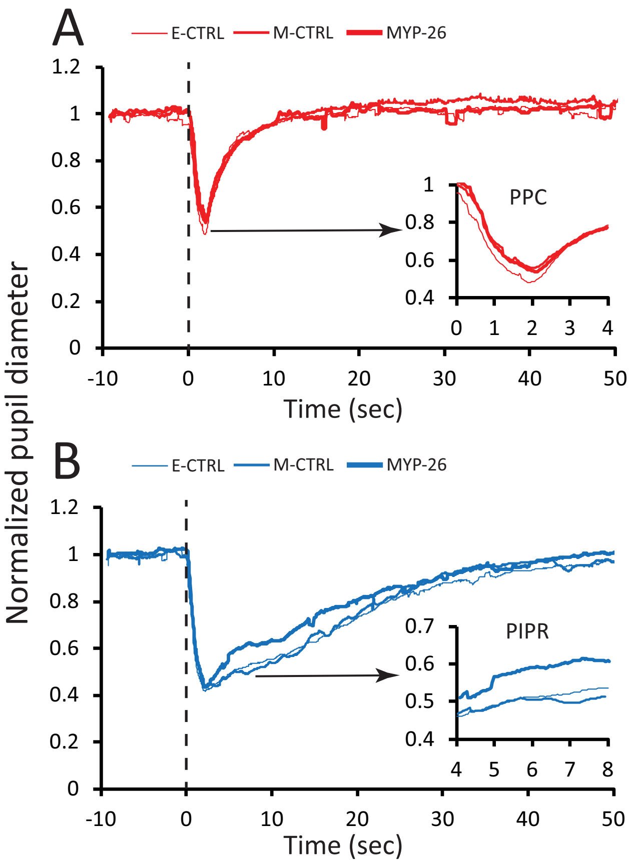

Figure 4. Averaged pupillary light reflex. The PLR traces to the red (**A**) and blue (**B**) flashes of averaged responses from each group: E-CTRL ($N = 24$ eyes, thin traces), M-CTRL ($N = 14$ eyes, traces with the intermediate thickness) and MYP-26 ($N = 14$ eyes, thicker traces). The PLR was characterized by a pupillary constriction briefly after light stimulation (*dotted lines* at time 0) followed by the pupil dilation after light offset. In the blue condition, the pupil redilation is slower originating from a sustained component. The smaller plots show the magnification of the peak pupil constriction (PPC) and the postillumination pupil response (PIPR) for the red and the blue conditions, respectively.

the differences were limited to MYP-26 compared to E-CTRL ($P < 0.001$) and compared to M-CTRL ($P = 0.003$), whereas PIPR6 was comparable between the control groups (E-CTRL × M-CTRL $P = 0.999$). Interestingly, the PIPR6 of the male carrier was comparable to PIPR6 from an age-matched male control (mean of the eyes = 0.51). The pupil constriction at PIPR6 was 0.55 (mean of the eyes) in both the test and retest. These PIPR6 values were similar to female E-CTRL PIPR6 = 0.51 ± 0.06 and female M-CTRL = 0.50 ± 0.05, but higher than MYP-26 PIPR6 (mean = 0.42 ± 0.07) values (see Table 2).

The reduced PIPR6 in patients with MYP-26 means that after the peak constriction, the pupil was more rapidly redilated. However, affected PIPR at 6 seconds after the blue flash offset was later recovered, considering that PIPR30 was comparable among the three groups (group effect: $F_{(2,49)} = 0.216$ and $P = 0.806$). The means are shown in Figure 5D and Table 2.

Figure 6 shows that there was no correlation between PIPR6 and age (M-CTRL: $r = -0.148$, $P = 0.613$ and MYP-26: $r = -0.069$, $P = 0.815$), SE (M-CTRL: $r = -0.005$, $P = 0.987$ and MYP-26: $r = 0.144$, $P = 0.623$), and BCVA (MYP-26: $r = -0.229$, $P = 0.431$).

Discussion

The PLR has its primary origin in retinal photoreceptors: rods expressing rhodopsin (*RHO* gene; sensitivity spectral peak between 490 and 500 nm), cones expressing opsins sensitive to short (*OPN1SW* gene; approximately 420 nm), middle (*OPN1MW* gene; approximately 530 nm), and long wavelengths (*OPN1LW*; approximately 560 nm)[33] and ipRGCs expressing melanopsin (*OPN4* gene; 482 nm).[28] The proportion of activation provided by each photoreceptor type depends on the light intensity[34] and the spectral distribution ("color") of the light.[31] The PIPR following the PPC is strongly affected by the spectral characteristics of the light. It has been well established that short-wavelength (blue) light may directly activate melanopsin expressing ipRGCs,[28,35] which is the neural substrate of the sustained PIPR component elicited by blue light at intensities of around 100 cd/m^2 and brighter.[24]

In the present study, PLRs recorded to long- and short-wavelength brief flashes of light revealed an association between weaker PIPR6 under blue-light condition and MYP-26. The possible pathomechanisms involved in the ipRGC-melanopsin system dysfunction and speculations regarding genotype to phenotype relationships are discussed below.

The first observation is that HM itself has little effect on PLRs elicited by red and blue brief flashes of light. This conclusion was achieved by comparing PLR results from patients with eoHM harboring intact *ARR3* alleles (the M-CTRL group) and the emmetropic controls (E-CTRL group). Here, we show (see Fig. 4A) that the PPC to the red flash may be slightly affected by HM. It is worth mentioning, that (i) the genetic background of the M-CTRL group is not known, and (ii) the present study used dark-adapted single flashes to record PLRs which differs from the method reported by Mutti et al.[36] to study refractive error effects on PLRs. In the latter work, the authors applied the repeated light exposure method, finding that the PLR changes under light-adaptation, presumably due to the modulation of melanopsin inputs by the repeated stimulation.[36] Nevertheless, completely preserved PIPR in patients with eoHM without *ARR3* gene mutations showed here corroborates previous studies consistently failing to find associations between mild to moderate myopia and PIPR.[32,37–40] To date, only one such study dealt with HM, showing that red and blue PLRs of a single patient with HM (SE = −9.25) were similar to that of emmetropes and patients with mild to moderate myopia.[32]

The second observation – with an exception of one patient with MYP-26 showing bilateral miosis, was that scotopic BPD recorded prior to flash onset was similar among the three groups: E-CTRL, M-CTRL, and MYP-26. This observation is congruent with previous findings of comparable pupil size in emmetropes and myopes, whereas it was significantly higher in myopes as compared to hyperopes.[41] Conversely, refractive status has been reported to impact light-adapted pupil size with the larger pupils associated with the myopic status.[40,42–44] In the present study, no associations were found between BPD and myopia, under the dark-adapted condition.

The main finding of the present study was the decrease of the sustained component of the PIPR to the blue flash,

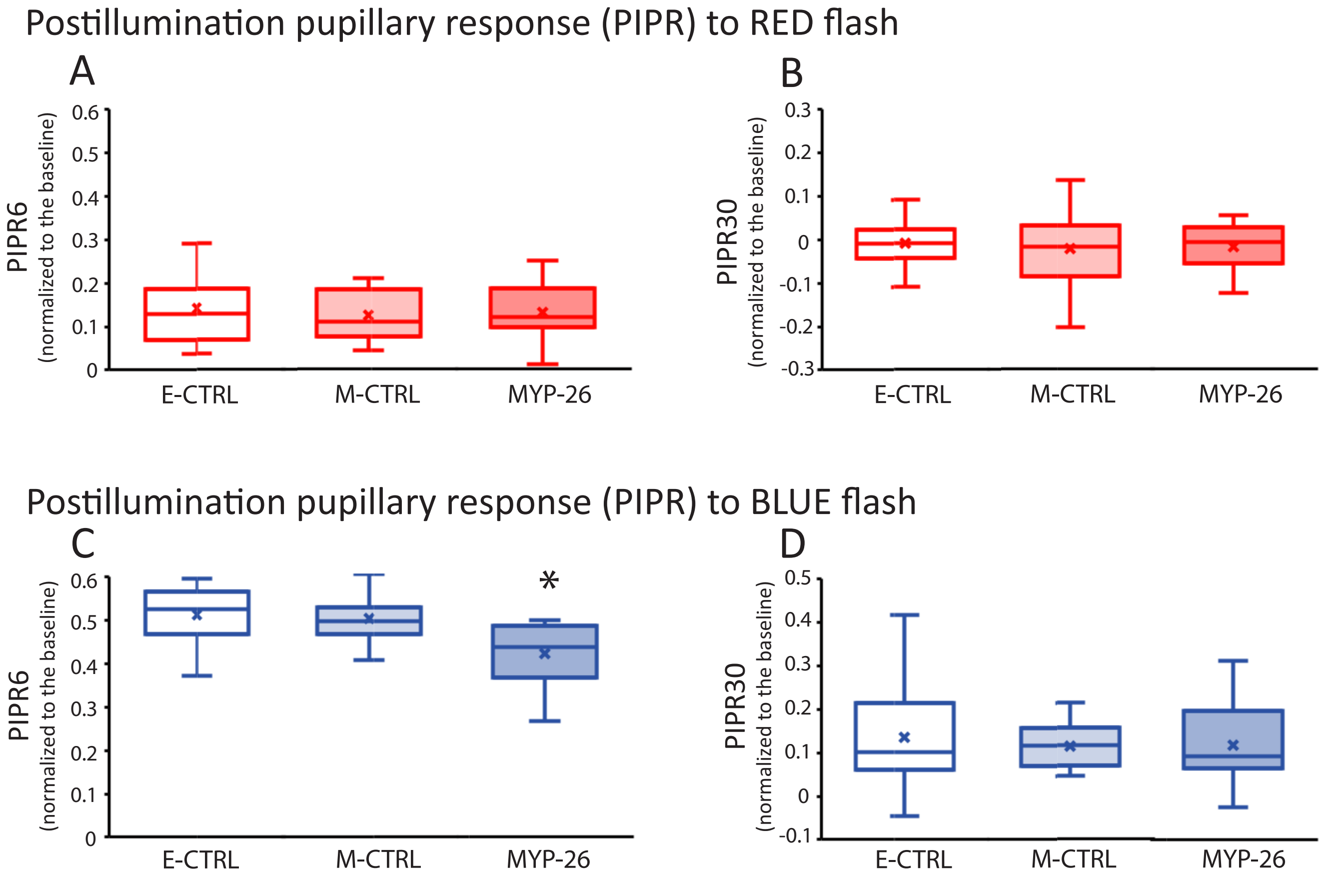

FIGURE 5. **Postillumination pupillary response (PIPR) results** for E-CTRL (*empty symbols*), M-CTRL (*light color*), and MYP-26 (*dark color*). (**A**) Mean PIPR6 to the red flash were similar among the groups. (**B**) Comparable PIPR30 to red flash was also observed. (**C**) The PIPR to the blue flash was significantly decreased for the MYP-26 group (marked with an *asterisk*) compared to both control groups E-CTRL and M-CTRL. (**D**) PIPR30 to the blue flashes were similar among the groups. * Significant ($P < 0.05$) values with post hoc Bonferroni test when compared to either control group (E-CTRL and M-CTRL).

indicating the weaker signal originating from the ipRGC-melanopsin system.[23] This was exclusively observed in *ARR3* heterozygous (MYP-26) female patients of the affected family (see Fig. 4B), evidencing that cone-arrestin is required for the proper functioning of the ipRGC-melanopsin system. Whether the disruption of PIPR is caused by dysfunctional outer retinal photoreceptors or it is due to circadian rhythms dysregulated by the altered cone-arrestin in MYP-26 retinas remain to be investigated. Normal PIPR6 to blue light in male carriers with hemizygous *ARR3* gene mutation remains to be further confirmed. However, the description of a single case in the present study suggests that ipRGC-melanopsin dysfunction might be restricted to female patients and, therefore, involved in the pathological eye growth of patients with MYP-26.

Photoreceptor dysfunction influencing the activity of the retinal ganglion cells (RGCs) in patients with MYP-26 was previously demonstrated by our group based on electrophysiological results showing reduced and delayed pattern electroretinogram (PERG).[3] Therefore, it could be argued that the altered PIPR6 to the blue flash reported here was simply the effect of a general RGC dysfunction. However, it is known that the contribution of ipRGCs to the PERG is not significant, as normal PERG was found in an experimental mouse model ($OPN4^{-/-}$) lacking melanopsin expression.[45] It is worth mentioning that reduced and delayed PERG amplitudes are biomarkers of moderate[46] and high[47] myopia, regardless of the genetic background, possibly caused by the pathological eye elongation disrupting ganglion cells' function in general. Still, this study found that patients with eoHM without the genetic alteration of the *ARR3* gene showed PIPRs comparable to emmetropes, contrary to what one would expect if the general RGC dysfunction would lead to a weakened ipRGC-melanopsin signal. Our previous study found PERG alterations (indicating a general RGC dysfunction) in both, female patients and male carriers.[3] In this study, a male carrier did not display an altered PIPR6, therefore, the generalized RGC dysfunction linked to this *ARR3* gene mutation does not seem to necessarily cause ipRGC dysfunction.

Nevertheless, the outer retinal photoreceptors certainly contribute to the PLR elicited by the red and blue flashes,[31] as shown in Figure 2B. However, rod as well as L/M-cone inputs alone contribute minimally to ipRGCs signaling neurons in the olivary pretectal nucleus (OPN), the subcortical substrate of PLR.[48] S-cones, on the other hand, are possible contributors of OPN/PLR activation[48] with a peak spectral sensitivity (420 nm) close to that of the melanopsin.[28] It has been recently shown that S-cone function is apparently preserved in patients with MYP-26,[4] which

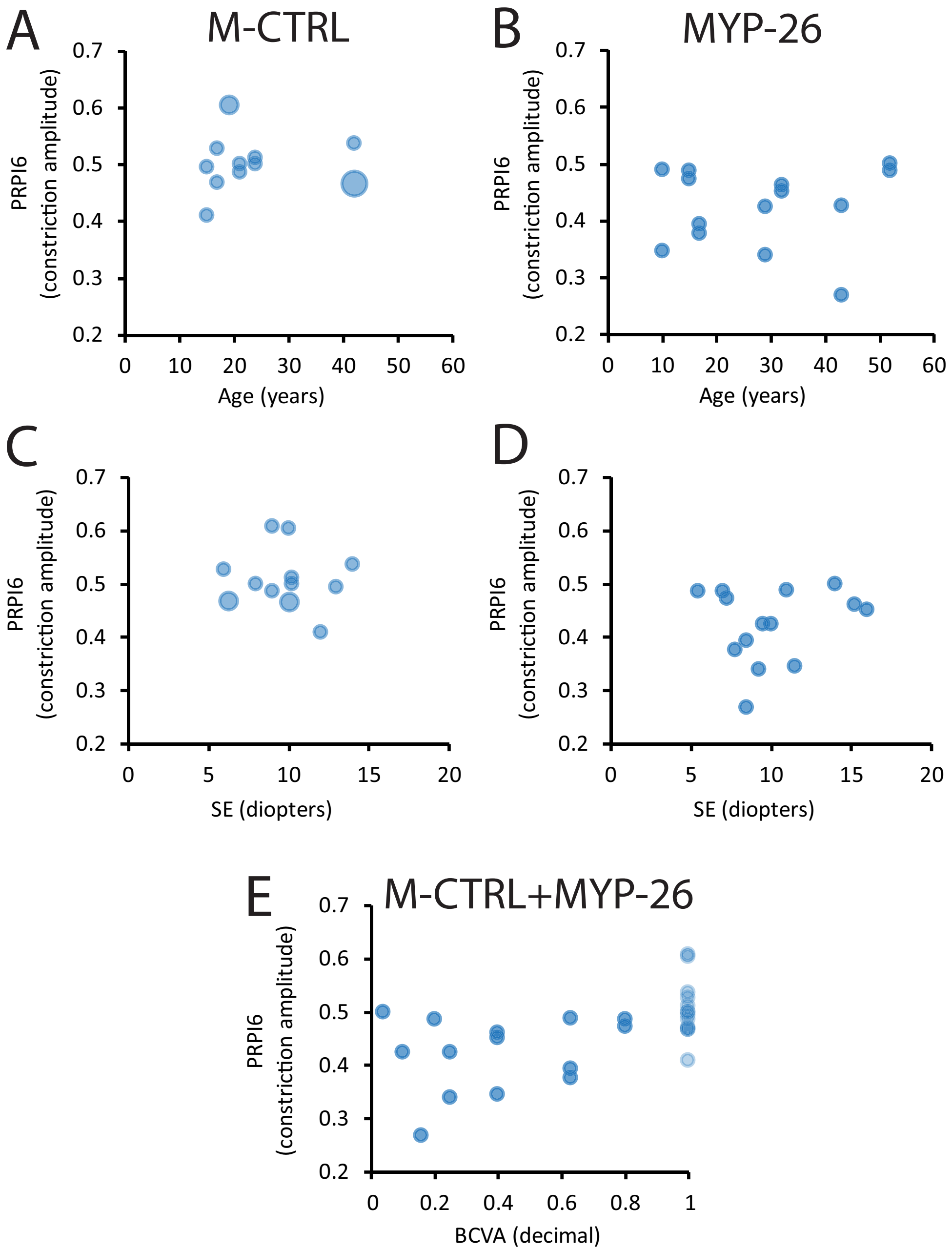

Figure 6. **Testing for correlation of PIPR6 (after the blue flash) and various parameters of M-CTRL and patients with MYP-26**. PIPR6 to the blue flash as a function of age (**A**) M-CTRL and (**B**) MYP-26, spherical equivalent (SE) (**C**) M-CTRL and (**D**) MYP-26 and best-corrected visual acuity (BCVA) (**E**) both myopic groups, pooled. There were no significant correlations in either test. Larger symbols (**A** and **C**) represent two overlapping points and the extra-large symbol (**A**) represents three overlapping points.

is expected considering that ARR1, the rod arrestin, is highly concentrated, even more expressed than ARR3, in S-cones.[49] In L/M-cones, on the other hand, ARR1 is much less expressed, whereas ARR3, which is highly concentrated in cone synaptic pedicles, is the main cone-arrestin in these cells[49] showing unique modulatory roles.[50] This could mean that S-cone dysfunction is less likely to lead to PIPR defects and, therefore, may indicate the direct effect of *ARR3* gene mutations on ipRGC-melanopsin system dysfunction.

A further question would be consequently whether the ipRGC-melanopsin system disruption suggested by the PLR changes in patients with MYP-26 stimulate pathological eye growth in female patients? It has been demonstrated that the ipRGC-melanopsin system is a key player in emmetropization.[14,19,51] Pathological eye growth and myopic shifts were observed in mouse models with disrupted melanopsin system with,[14] but also without[15] a functional rod and cone system. Moreover, under form-deprivation conditions, the proper activation of the ipRGC-melanopsin system is required to slow myopic progression. The melanopsin system dysfunction was associated to three times higher myopic shifts in mice.[14] Interestingly, ipRGCs provide excitatory inputs to dopaminergic amacrine cells,[52] probably the only cell type synthetizing dopamine in the retina.[53] This is most likely the mechanism underlying melanopsin signaling-related form-deprivation myopic development.[14] It became evident that the normal levels of retinal dopamine is essential for the postnatal regulation of eye growth.[54,55] The lower the dopamine level the higher the susceptibility to develop form-deprivation[56,57] and spontaneous[58] myopia. In addition, it has been demonstrated in animal models that increasing dopamine levels in the retina by genetic or pharmacological manipulation inhibits myopia development.[54] In fact, dopamine is involved in both experimentally induced and spontaneous myopia (for a review, see Feldkaemper and Schaeffel 2013[59]) and the administration of levodopa inhibits the development of form-deprivation[60,61] and lens-induced[62] myopia by modulating dopamine D2-like receptor, mainly DRD4 dopamine receptor. Through retinal volume transmission, dopamine interacts with DRD4 dopaminergic receptors highly expressed in photoreceptors and from which desensitization/internalization is controlled by cone-arrestin, in addition to beta-arrestin.[10] Photoreceptor DRD4 dopamine receptors have been proposed to influence photoreceptor function,[63] retinal light-adaptation,[64] electrical coupling between rods and cones, especially under mesopic light condition,[65] and retinal melatonin synthesis.[66,67] These are all DRD4/dopamine-dependent mechanisms related to visual conditions known to impact emmetropization in humans.[68–72] Although outer retinal photoreceptors are the main modulators of the dopamine pathway, ipRGC-melanopsin system certainly influences the retinal levels of dopamine.[57]

An overall speculation accordingly could be that the mechanisms of (i) cone dysfunction causing form-deprivation/retinal defocus, as recently proposed elsewhere,[4] and (ii) the alteration in the ipRGC-melanopsin system shown here, which is known to affect the dopaminergic pathways and, consequently, eye growth,[73] may both impact the pathogenesis of MYP-26. Further investigations are required to evaluate whether the ipRGC-melanopsin system dysfunction introduces a complex or multifactorial phenomena to the pathological eye growth in MYP-26 and possibly to myopization in other inherited retinal diseases. Our study is limited to seven affected women and one carrier male, all related. PLR measurements should therefore be extended to patients with MYP-26 independent of the family investigated here to verify the general nature of our findings. Nevertheless, the recently proposed cone dysfunction stimulating eoHM in female patients[4] could be further re-interpreted in the light of retinal circadian rhythms influencing myopia development.[51,74]

As reported earlier by our team[75] and other groups,[76] the ipRGC-melanopsin system dysfunction is associated with sleep disturbances. Therefore, PIPR alterations in patients with MYP-26 could be associated with sleep symptoms which was, nevertheless, not the case in our patients. However, an important remark regarding the present data is that ipRGC-melanopsin system driven PIPR is disrupted but not completely suppressed in MYP-26. It is rather able to reach full pupil redilation at 30 seconds after flash offset, which is comparable to emmetropes and patients with eoHM without *ARR3* gene mutations.

The present results emphasize that PLR is a powerful noninvasive technique allowing the objective measurement of retinal cells' integrity.[23,27,77]

CONCLUSIONS

We detected weaker sustained postillumination pupil constriction in female patients with MYP-26, but not in the male carrier of the *ARR3* gene mutation. This finding indicates the dysfunction of the ipRGC-melanopsin system exclusively in patients with myopia, which may act as a further pathomechanism of pathological eye elongation, in addition to the previously reported L/M cone dysfunction.[4]

Acknowledgments

Supported by the National Research, Development, and Innovation Fund of Hungary to MTSB (NKFI OTKA-PD grant number 134799); Tempus Public Foundation (Hungary) to MTSB (TKA grant number 167949), and the Biotechnology National Laboratory of Hungary to T.F.

Disclosure: **M.T.S. Barboni**, None; **N. Széll**, None; **Z. Sohajda**, None; **T. Fehér**, None

References

1. Xiao X, Li S, Jia X, Guo X, Zhang Q. X-linked heterozygous mutations in ARR3 cause female-limited early onset high myopia. *Mol Vis*. 2016;22:1257–1266.
2. van Mazijk R, Haarman AEG, Hoefsloot LH, et al. Early onset X-linked female limited high myopia in three multigenerational families caused by novel mutations in the ARR3 gene. *Hum Mutat*. 2022;43(3):380–388.
3. Széll N, Fehér T, Maróti Z, et al. Myopia-26, the female-limited form of early-onset high myopia, occurring in a European family. *Orphanet J Rare Dis*. 2021;16(1):45.
4. Gu L, Cong P, Ning Q, Jiang B, Wang J, Cui H. The causal mutation in ARR3 gene for high myopia and progressive color vision defect. *Sci Rep*. 2023;13(1):8986.
5. Wang Y, Xiao X, Li X, et al. Genetic and clinical landscape of ARR3-associated MYP26: the most common cause of Mendelian early-onset high myopia with a unique inheritance. *Br J Ophthalmol*. Published online September 30, 2022: bjophthalmol-2022-321511. doi:10.1136/bjo-2022-321511.
6. Xiao X, Yang J, Li Y, et al. Identification of a novel frameshift variant of ARR3 related to X-Linked female-limited early-onset high myopia and study on the effect of X chromosome inactivation on the myopia severity. *J Clin Med*. 2023;12(3):835.
7. Deming JD, Pak JS, Brown BM, et al. Visual cone arrestin 4 contributes to visual function and cone health. *Invest Ophthalmol Vis Sci*. 2015;56(9):5407–5416.
8. Craft CM, Whitmore DH, Wiechmann AF. Cone arrestin identified by targeting expression of a functional family. *J Biol Chem*. 1994;269(6):4613–4619.
9. Smith WC. The role of arrestins in visual and disease processes of the eye. *Prog Mol Biol Transl Sci*. 2013;118:243–265.
10. Deming JD, Shin JA, Lim K, Lee EJ, Van Craenenbroeck K, Craft CM. Dopamine receptor D4 internalization requires a beta-arrestin and a visual arrestin. *Cell Signal*. 2015;27(10):2002–2013.
11. Bhoi JD, Goel M, Ribelayga CP, Mangel SC. Circadian clock organization in the retina: from clock components to rod and cone pathways and visual function. *Prog Retin Eye Res*. Published online December 8, 2022: 101119. doi:10.1016/j.preteyeres.2022.101119.
12. Zhang S, Lyuboslavsky P, Dixon JA, et al. Effects of cone connexin-36 disruption on light adaptation and circadian

regulation of the photopic ERG. *Invest Ophthalmol Vis Sci*. 2020;61(6):24.

13. Dkhissi-Benyahya O, Coutanson C, Knoblauch K, et al. The absence of melanopsin alters retinal clock function and dopamine regulation by light. *Cell Mol Life Sci*. 2013;70(18):3435–3447.
14. Chakraborty R, Landis EG, Mazade R, et al. Melanopsin modulates refractive development and myopia. *Exp Eye Res*. 2022;214:108866.
15. Liu AL, Liu YF, Wang G, et al. The role of ipRGCs in ocular growth and myopia development. *Sci Adv*. 2022;8(23):eabm9027.
16. Ashby R, Ohlendorf A, Schaeffel F. The effect of ambient illuminance on the development of deprivation myopia in chicks. *Invest Ophthalmol Vis Sci*. 2009;50(11):5348–5354.
17. Smith EL, Hung LF, Huang J. Protective effects of high ambient lighting on the development of form-deprivation myopia in rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2012;53(1):421–428.
18. Wang M, Schaeffel F, Jiang B, Feldkaemper M. Effects of light of different spectral composition on refractive development and retinal dopamine in chicks. *Invest Ophthalmol Vis Sci*. 2018;59(11):4413–4424.
19. Chakraborty R, Ostrin LA, Nickla DL, Iuvone PM, Pardue MT, Stone RA. Circadian rhythms, refractive development, and myopia. *Ophthalmic Physiol Opt*. 2018;38(3):217–245.
20. Read SA, Collins MJ, Vincent SJ. Light exposure and eye growth in childhood. *Invest Ophthalmol Vis Sci*. 2015;56(11):6779–6787.
21. Morgan IG, Wu PC, Ostrin LA, et al. IMI risk factors for myopia. *Invest Ophthalmol Vis Sci*. 2021;62(5):3.
22. Hysi PG, Choquet H, Khawaja AP, et al. Meta-analysis of 542,934 subjects of European ancestry identifies new genes and mechanisms predisposing to refractive error and myopia. *Nat Genet*. 2020;52(4):401–407.
23. Adhikari P, Zele AJ, Feigl B. The post-illumination pupil response (PIPR). *Invest Ophthalmol Vis Sci*. 2015;56(6):3838–3849.
24. Gamlin PDR, McDougal DH, Pokorny J, Smith VC, Yau KW, Dacey DM. Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vision Res*. 2007;47(7):946–954.
25. Kankipati L, Girkin CA, Gamlin PD. Post-illumination pupil response in subjects without ocular disease. *Invest Ophthalmol Vis Sci*. 2010;51(5):2764–2769.
26. Kelbsch C, Strasser T, Chen Y, et al. Standards in pupillography. *Front Neurol*. 2019;10:129.
27. Park JC, Moura AL, Raza AS, Rhee DW, Kardon RH, Hood DC. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. *Invest Ophthalmol Vis Sci*. 2011;52(9):6624–6635.
28. Dacey DM, Liao HW, Peterson BB, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature*. 2005;433(7027):749–754.
29. Ohno-Matsui K. Pathologic myopia. *Ann Eye Sci*. 2018;3(2):8.
30. Asakawa K, Imai M, Ohta M, Kawata N, Kawatsu N, Ishikawa H. Pupil assessment with a new handheld pupillometer in healthy subjects. *Int Ophthalmol*. 2023;43(1):51–61.
31. Kardon R, Anderson SC, Damarjian TG, Grace EM, Stone E, Kawasaki A. Chromatic pupil responses: preferential activation of the melanopsin-mediated versus outer photoreceptor-mediated pupil light reflex. *Ophthalmology*. 2009;116(8):1564–1573.
32. Adhikari P, Pearson CA, Anderson AM, Zele AJ, Feigl B. Effect of age and refractive error on the melanopsin mediated post-illumination pupil response (PIPR). *Sci Rep*. 2015;5:17610.
33. Dartnall HJ, Bowmaker JK, Mollon JD. Human visual pigments: microspectrophotometric results from the eyes of seven persons. *Proc R Soc Lond B Biol Sci*. 1983;220(1218):115–130.
34. Ellis CJ. The pupillary light reflex in normal subjects. *Br J Ophthalmol*. 1981;65(11):754–759.
35. Panda S, Nayak SK, Campo B, Walker JR, Hogenesch JB, Jegla T. Illumination of the melanopsin signaling pathway. *Science*. 2005;307(5709):600–604.
36. Mutti DO, Mulvihill SP, Orr DJ, Shorter PD, Hartwick ATE. The effect of refractive error on melanopsin-driven pupillary responses. *Invest Ophthalmol Vis Sci*. 2020;61(12):22.
37. Abbott KS, Queener HM, Ostrin LA. The ipRGC-driven pupil response with light exposure, refractive error, and sleep. *Optom Vis Sci*. 2018;95(4):323–331.
38. Ansari AS, Vehof J, Hammond CJ, Bremner FD, Williams KM. Evidence that pupil size and reactivity are determined more by your parents than by your environment. *Front Neurol*. 2021;12:651755.
39. Ostrin LA. The ipRGC-driven pupil response with light exposure and refractive error in children. *Ophthalmic Physiol Opt*. 2018;38(5):503–515.
40. Rukmini AV, Chew MC, Finkelstein MT, et al. Effects of low and moderate refractive errors on chromatic pupillometry. *Sci Rep*. 2019;9(1):4945.
41. Lee YS, Kim HJ, Lim DK, Kim MH, Lee KJ. Age-specific influences of refractive error and illuminance on pupil diameter. *Medicine*. 2022;101(27):e29859.
42. Guillon M, Dumbleton K, Theodoratos P, Gobbe M, Wooley CB, Moody K. The effects of age, refractive status, and luminance on pupil size. *Optom Vis Sci*. 2016;93(9):1093–1100.
43. Hirsch MJ, Weymouth FW. Pupil size in ametropia. *J Appl Physiol*. 1949;1(9):646–648.
44. Winn B, Whitaker D, Elliott DB, Phillips NJ. Factors affecting light-adapted pupil size in normal human subjects. *Invest Ophthalmol Vis Sci*. 1994;35(3):1132–1137.
45. Milla-Navarro S, Pazo-González M, Germain F, de la Villa P. Phenotype characterization of a mice genetic model of absolute blindness. *Int J Mol Sci*. 2022;23(15):8152.
46. Hidajat R, Mclay J, Burley C, Elder M, Morton J, Goode D. Influence of axial length of normal eyes on PERG. *Doc Ophthalmol*. 2003;107(2):195–200.
47. Flitcroft DI, Adams GGW, Robson AG, Holder GE. Retinal dysfunction and refractive errors: an electrophysiological study of children. *Br J Ophthalmol*. 2005;89(4):484–488.
48. Allen AE, Brown TM, Lucas RJ. A distinct contribution of short-wavelength-sensitive cones to light-evoked activity in the mouse pretectal olivary nucleus. *J Neurosci*. 2011;31(46):16833–16843.
49. Nikonov SS, Brown BM, Davis JA, et al. Mouse cones require an arrestin for normal inactivation of phototransduction. *Neuron*. 2008;59(3):462–474.
50. Deming JD, Pak JS, Shin JA, et al. Arrestin 1 and cone arrestin 4 have unique roles in visual function in an all-cone mouse retina. *Invest Ophthalmol Vis Sci*. 2015;56(13):7618–7628.
51. Stone RA, Pardue MT, Iuvone PM, Khurana TS. Pharmacology of myopia and potential role for intrinsic retinal circadian rhythms. *Exp Eye Res*. 2013;114:35–47.
52. Zhang DQ, Wong KY, Sollars PJ, Berson DM, Pickard GE, McMahon DG. Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. *Proc Natl Acad Sci USA*. 2008;105(37):14181–14186.
53. Raja S, Milosavljevic N, Allen AE, Cameron MA. Burning the candle at both ends: Intraretinal signaling of intrinsically

photosensitive retinal ganglion cells. *Front Cell Neurosci*. 2022;16:1095787.

54. Landis EG, Chrenek MA, Chakraborty R, et al. Increased endogenous dopamine prevents myopia in mice. *Exp Eye Res*. 2020;193:107956.
55. Stone RA, Lin T, Laties AM, Iuvone PM. Retinal dopamine and form-deprivation myopia. *Proc Natl Acad Sci USA*. 1989;86(2):704–706.
56. Chakraborty R, Pardue MT. Chapter Fifteen - Molecular and biochemical aspects of the retina on refraction. In: Hejtmancik JF, Nickerson JM, eds. *Progress in Molecular Biology and Translational Science*. Vol 134. Molecular Biology of Eye Disease. San Diego, CA: Academic Press; 2015:249–267.
57. Zhou X, Pardue MT, Iuvone PM, Qu J. Dopamine signaling and myopia development: what are the key challenges. *Prog Retin Eye Res*. 2017;61:60–71.
58. Bergen MA, Park HN, Chakraborty R, et al. Altered refractive development in mice with reduced levels of retinal dopamine. *Invest Ophthalmol Vis Sci*. 2016;57(10):4412–4419.
59. Feldkaemper M, Schaeffel F. An updated view on the role of dopamine in myopia. *Exp Eye Res*. 2013;114:106–119.
60. Thomson K, Karouta C, Morgan I, Kelly T, Ashby R. Effectiveness and safety of topical levodopa in a chick model of myopia. *Sci Rep*. 2019;9(1):18345.
61. Ward AH, Siegwart JT, Frost MR, Norton TT. Intravitreally-administered dopamine D2-like (and D4), but not D1-like, receptor agonists reduce form-deprivation myopia in tree shrews. *Vis Neurosci*. 2017;34:E003.
62. Thomson K, Morgan I, Karouta C, Ashby R. Levodopa inhibits the development of lens-induced myopia in chicks. *Sci Rep*. 2020;10(1):13242.
63. Jackson CR, Chaurasia SS, Zhou H, Haque R, Storm DR, Iuvone PM. Essential roles of dopamine D4 receptors and the type 1 adenylyl cyclase in photic control of cyclic AMP in photoreceptor cells. *J Neurochem*. 2009;109(1):148–157.
64. Nir I, Harrison JM, Haque R, et al. Dysfunctional light-evoked regulation of cAMP in photoreceptors and abnormal retinal adaptation in mice lacking dopamine D4 receptors. *J Neurosci*. 2002;22(6):2063–2073.
65. Krizaj D. Mesopic state: cellular mechanisms involved in pre- and post-synaptic mixing of rod and cone signals. *Microsc Res Tech*. 2000;50(5):347–359.
66. Klitten LL, Rath MF, Coon SL, Kim JS, Klein DC, Møller M. Localization and regulation of dopamine receptor D4 expression in the adult and developing rat retina. *Exp Eye Res*. 2008;87(5):471–477.
67. Zawilska JB, Nowak JZ. Does D4 dopamine receptor mediate the inhibitory effect of light on melatonin biosynthesis in chick retina? *Neurosci Lett*. 1994;166(2):203–206.
68. Flanagan SC, Cobice D, Richardson P, Sittlington JJ, Saunders KJ. Elevated melatonin levels found in young myopic adults are not attributable to a shift in circadian phase. *Invest Ophthalmol Vis Sci*. 2020;61(8):45.
69. Jiang X, Xu Z, Soorma T, et al. Electrical responses from human retinal cone pathways associate with a common genetic polymorphism implicated in myopia. *Proc Natl Acad Sci USA*. 2022;119(21):e2119675119.
70. Landis EG, Yang V, Brown DM, Pardue MT, Read SA. Dim light exposure and myopia in children. *Invest Ophthalmol Vis Sci*. 2018;59(12):4804–4811.
71. Williams KM, Georgiou M, Kalitzeos A, et al. Axial length distributions in patients with genetically confirmed inherited retinal diseases. *Invest Ophthalmol Vis Sci*. 2022;63(6):15.
72. Zeitz C, Roger JE, Audo I, et al. Shedding light on myopia by studying complete congenital stationary night blindness. *Prog Retin Eye Res*. 2023;93:101155.
73. Landis EG, Park HN, Chrenek M, et al. Ambient light regulates retinal dopamine signaling and myopia susceptibility. *Invest Ophthalmol Vis Sci*. 2021;62(1):28.
74. Stone RA, McGlinn AM, Chakraborty R, et al. Altered ocular parameters from circadian clock gene disruptions. *PLoS One*. 2019;14(6):e0217111.
75. Barboni MTS, Bueno C, Nagy BV, et al. Melanopsin system dysfunction in Smith-Magenis syndrome patients. *Invest Ophthalmol Vis Sci*. 2018;59(1):362–369.
76. Feigl B, Dumpala S, Kerr GK, Zele AJ. Melanopsin cell dysfunction is involved in sleep disruption in Parkinson's disease. *J Parkinsons Dis*. 2020;10(4):1467–1476.
77. Barrionuevo PA, Issolio LA, Tripolone C. Photoreceptor contributions to the human pupil light reflex. *J Photochem Photobiol*. 2023;15:100178.