Association Between Postillumination Pupil Response and Glaucoma Severity: A Cross-Sectional Analysis of the LIGHT Study

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Purpose. This study determines whether the functional and structural severity of glaucoma is associated with intrinsically photosensitive retinal ganglion cell (ipRGC) function.

Methods. This cross-sectional study assessed 148 eyes from 148 patients with glaucoma (mean age 70.5 years). The ipRGC function was assessed by postillumination pupil response (PIPR) using the pupil diameter after exposure to blue and red light. Main outcome measures were as follows: six-second PIPR amplitude, net PIPR, and net PIPR change. Functional and structural glaucoma severities were evaluated using visual field mean deviation (MD) and the circumpapillary retinal nerve fiber layer (RNFL) thickness, respectively.

Results. Multivariable analysis adjusting for age, sex, body mass index, hypertension, diabetes, oral medication use, cataract surgery, axial length, and topical alpha$_2$-adrenergic receptor agonist use showed that worsening in visual field MD was significantly associated with higher blue six-second PIPR amplitude (regression coefficient per −1 dB worsening, 0.25; 95% confidence intervals [CI], 0.14, 0.37; $P < 0.001$). The thinner RNFL thickness was significantly associated with higher blue six-second PIPR amplitude, lower Net PIPR change, and lower net PIPR (blue six-second PIPR amplitude: regression coefficient per 10-µm thinning, 1.29; 95% CI, 0.72, 1.87; $P < 0.001$; net PIPR change: regression coefficient, −0.70; 95% CI, −1.26, −0.14; $P = 0.015$; net PIPR: regression coefficient, −0.03; 95% CI, −0.05, −0.001; $P = 0.044$). No significant association was found between glaucoma severity and red six-second PIPR amplitude.

Conclusions. Our findings revealed a significant association between functional and structural glaucoma severity and impaired ipRGC function independent of potential confounders.

Keywords: glaucoma, post-illumination pupil response, ipRGCs, intrinsically photosensitive retinal ganglion cells, circadian rhythm

Glaucoma, which is characterized by progressive retinal ganglion cell (RGC) death, remains the most prevalent cause of irreversible blindness worldwide.[1] Moreover, studies have shown that glaucoma is associated with the disruption of the circadian biological rhythm given its effects on melatonin secretion, sleep, mood disorder, cognitive impairment, and nighttime blood pressure.[2–7] The circadian biological rhythm is regulated in the suprachiasmatic nuclei, which is known as the circadian master clock, through light reception in the retina, particularly in intrinsically photosensitive retinal ganglion cells (ipRGCs).[8] A histological study showed reduced ipRGC density in human donor eyes with severe glaucoma.[9] Thus the loss of ipRGCs in patients with glaucoma may disrupt the circadian biological rhythm.

The ipRGCs are unique photosensitive cells containing photopigment melanopsin that are morphologically and functionally distinct from the other classic photoreceptors, such as rods and cones. The ipRGCs, which have large somas and dendrites, account for approximately 0.3% of the total RGCs.[10,11] The physiological function of ipRGCs is to transmit the non–image-forming light to the suprachiasmatic nuclei for the entrainment of the circadian biological rhythms.[8] Moreover, ipRGCs exhibit projection to the olivary pretectal nucleus in the midbrain and are involved in the pupillary light reflex through the reception of the short wavelength blue light, particularly at approximately 480 nm. The pupillary light reflex mediated by ipRGCs is characterized by sustained constriction and slow recovery after blue light stimulus offset.[12,13] Given the aforementioned characteristics of pupillary light reflex after blue light stimulus, ipRGC function has been evaluated as the postillumination pupil response (PIPR) in patients with various neurological disorders.[14,15]

Earlier clinical studies (n = 25–46) have reported that glaucomatous damages, including worsening visual field mean deviation (MD) and thinning retinal nerve fiber layer

(RNFL), were correlated with impaired ipRGC function evaluated through pupillary light reflex, including PIPR.[14,16–18] Several basic and clinical factors, such as age, sex, diabetes, oral medication use, topical alpha$_2$-adrenergic receptor agonist use, cataract surgery, and refractive error, can reportedly affect pupillary light reflex including PIPR.[19–22] A previous univariable analysis, which excluded patients with some potential confounders, revealed that glaucoma severity is associated with PIPR; however, no evidence was obtained by multivariable analysis adjusted for various potential confounders.[14,16–18] A study with a reliably large sample size is required to enable a multivariable analysis adjusted for various potential confounders.

We believe that the reliable investigation of association between glaucoma and impaired ipRGC function is essential to elucidate the mechanism of the influence of glaucoma on circadian disruption. Therefore the current cross-sectional study aimed to determine whether functional and structural glaucomatous damage, evaluated using visual field MD and RNFL thickness, was associated with ipRGC function, evaluated using PIPR, in the multivariable analysis of a large cohort comprising 148 patients with glaucoma.

Methods

Study Patients

Between in May 2017 and September 2020, 172 patients with glaucoma were enrolled in the "Longitudinal study of biological circadian rhythms In Glaucoma patients: Home Testing of circadian intraocular pressure and biological parameters" (LIGHT study).[7] All patients with severe corneal and retinal diseases that affected retinal visibility and ophthalmic evaluations of optic disc were excluded from the LIGHT study. Among the 172 patients, 24 were excluded from analyses for the following reasons: (1) missing measurement data of the pupillary light reflex (two patients); (2) interrupted measurement data of the pupillary light reflex (three patients); (3) unstable pupil, defined as an irregular and large amplitude fluctuation >0.5 mm in the pupil diameter during pupillary light reflex measurement (17 patients) (see Supplementary Fig. S1); and (4) unreliable pupillary light reflex defined as a pupil diameter after light stimulus that was greater than 10% of the baseline pupil diameter before light stimulus based on the exclusion of an earlier study (two patients).[18] Finally, 148 eyes from 148 patients with glaucoma were included in the analyses of the present study. In cases with bilateral glaucoma, the eye with more severe glaucoma was analyzed. The LIGHT study was approved by the Ethics Committee of Nara Medical University (approval number 1314) and was registered with the University Hospital Medical Information Network Clinical Trials Registry (registration number UMIN000027299). This protocol adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all glaucoma patients who participated in the LIGHT study.

All patients with glaucoma underwent complete ophthalmic assessment that included slit-lamp biomicroscopy, indirect ophthalmoscopy, gonioscopy, best-corrected visual acuity, intraocular pressure using Goldmann applanation tonometry, RNFL thickness using spectral-domain optical coherence tomography (Spectralis; Heidelberg Engineering, Heidelberg, Germany), and visual field evaluations using standard automated perimetry. Glaucoma was diagnosed by one glaucoma specialist (T.Y.) based on the presence of glaucomatous optic disc and concomitant glaucomatous visual field defects as described in detail previously.[7] Among the 148 patients with glaucoma, 107 (72.3%) had bilateral glaucoma, and 41 (27.7%) had unilateral glaucoma. Among the 148 eyes analyzed, 117 (79.1%) had primary open-angle glaucoma, 19 (12.8%) had secondary glaucoma including exfoliation glaucoma, and 12 (8.1%) had primary angle-closure glaucoma.

Visual Field Examinations

Visual field examinations for the evaluation of functional glaucoma severity and classification of glaucoma severity were conducted using the Humphrey Field Analyzer II (Humphrey; Carl Zeiss Meditec, Dublin, CA). The visual field MD was determined using the 30-2 Swedish Interactive Threshold Algorithm (SITA) standard program. Visual field MD data with a false-positive response >15% were excluded from statistical analyses based on an earlier study.[23] In cases with unreliable visual field data with a false-positive response >15%, reliable visual field data (n = 10) were substituted using SITA fast program for SITA standard program during our analyses. We divided the eyes with glaucoma into two groups (severe and nonsevere) according to the following criteria for severe glaucoma: (1) visual field MD ≤ -12 dB, (2) > 50% of the points are depressed below the 5% levels or > 20 points are depressed below the 1% levels on the pattern deviation plot, (3) at least one point in the central 5° has a sensitivity value of 0 dB, or (4) points within the central 5° have a sensitivity value of <15 dB in both hemifields.[24] Among the 148 patients, two with unmeasurable visual field data caused by the central scotoma were categorized into the severe glaucoma group based on criteria established in an earlier study.[25] Worsening in visual field MD was indicated by a more negative value.

Assessment of Circumpapillary RNFL Thickness

To evaluate the structural glaucoma severity, circumpapillary RNFL thickness was measured using the spectral-domain optical coherence tomography (Spectralis; Heidelberg Engineering). The RNFL scan program was used 1536 A-scans × 1 B-scan in a circular diameter of 3.5 mm centered on the optic disc. We used the global RNFL thickness for statistical analyses. All scan images were confirmed by one glaucoma specialist (T.Y.) to ensure the reliability of segmentation. Among the 148 eyes analyzed herein, 15 with unreliable RNFL thickness data, such as those with segmentation errors and a quality score $\leq$ 15, were excluded based on the results of an earlier study.[26] Finally, the association between structural glaucoma severity and PIPR parameters were analyzed in 133 eyes.

Pupillometer for the Measurement of the Pupillary Light Reflex

To evaluate ipRGC function, PIPR was measured by determining the pupillary light reflex using the RAPDx (Konan Medical USA, Inc., Irvine, CA, USA), a pupillometer designed to objectively measure the magnitude of the relative afferent pupillary defect. The RAPDx was programed to measure the PIPR based on methods used in an earlier study.[12] For ipRGC

excitation, we performed the monochromatic stimulus with approximately 25° of effective field of view using blue light with a 448-nm peak wavelength and irradiance of 2.70 × 10^{12} photons/s/cm^2 measured using spectroradiometer (PR-670; Photoresearch/JADAK Inc., North Syracuse, NY, USA). Moreover, we used red light with a 608-nm peak wavelength and irradiance of 2.58 × 10^{12} photons/s/cm^2 to evaluate the outer retina and serve as control. The pupil diameter was recorded at a frame rate of 40 Hz.

PIPR Measurement Protocol

The eye with more severe glaucoma was dilated using an eye drop containing 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P; Santen Pharmaceutical, Osaka, Japan) except for 12 eyes with primary angle-closure glaucoma. The dilated eye with more severe glaucoma was stimulated by red and blue light. Then, the PIPR parameters of the fellow eye without mydriasis were evaluated during pupillary light reflex measurement. PIPR was measured using the following protocol based on the methods utilized in an earlier study[12,13]: (1) Dark adaptation using an eye mask in a dark room was performed for five minutes. (2) After dark adaptation, the baseline pupil diameter was recorded for a duration of seven seconds before red light stimulus onset. (3) The initial pupil diameter after red light stimulus onset was recorded for a duration of 10 seconds. (4) After red light stimulus offset, red PIPR parameters were recorded for a duration of 40 seconds. (5) Once again, we performed dark adaptation for five minutes after measuring the red PIPR. (6) The baseline pupil diameter was recorded for a duration of seven seconds duration before blue light stimulus onset. (7) The initial pupil diameter following blue light stimulus onset was recorded for a duration of 10 seconds. (8) After blue light stimulus offset, the blue PIPR parameters were recorded for a duration of 40 seconds. All PIPR measurements were performed during afternoon from 1 PM to 4 PM to avoid the influence of circadian variations. The PIPR parameters (six-second PIPR amplitude,[13] Net PIPR change,[12] and Net PIPR[12]) were defined as follows:

Blue six-second PIPR amplitude (%) = [pupil diameter at six seconds after blue light stimulation offset (mm)/baseline pupil diameter (mm)] × 100

Red six-second PIPR amplitude (%) = [pupil diameter at six seconds after red light stimulation offset (mm)/baseline pupil diameter (mm)] × 100

Net PIPR change (%) = Blue sustained PIPR change (%) − Red sustained PIPR change (%)

Net PIPR (mm) = Blue sustained PIPR (mm) − Red sustained PIPR (mm)

We calculated sustained PIPR (mm) using the following formula: baseline pupil diameter (mm) − mean pupil diameter for a duration of 30 seconds starting from 10 seconds after light stimulus offset to 40 seconds (mm). Sustained PIPR change (%) was calculated by the following formula: (sustained PIPR/baseline pupil diameter) × 100.

Higher blue six-second PIPR amplitude, lower Net PIPR change and lower Net PIPR indicated lower ipRGC function given that the characteristics of the pupillary light reflex mediated by the ipRGCs, that is, sustained constriction and slower recovery after blue light stimulus offset. The representative pupillary light reflex was shown for patients with nonsevere glaucoma and severe glaucoma (Fig. 1).

Measurement of Covariates

We calculated the body mass index by dividing the patients' weight by the square of their height. The presence of hypertension was determined based on the patients' medical history and use of antihypertension drugs. Diabetes mellitus was defined based on current diabetes treatment, fasting plasma glucose ≥126 mg/dL or a glycated hemoglobin level ≥6.5%. The use of oral antihistamines and dopaminergic medications were determined based on the patients' medical history. None of the patients used the following oral medications that could potentially interfere with the pupillary light reflex: antihypertensives (prazosin and clonidine), antiarrhythmics, antidepressants, antipsychotics, psychostimulants, and antiemetics.[19] Cataract surgery was determined based on clinical assessment. Axial length was measured using partial coherence laser interferometry (IOL master; Carl Zeiss Meditec, Inc.) in the stimulated eye. The use of topical alpha$_2$-adrenergic receptor agonist (brimonidine) was determined based on the patients' medical history. None of the patients used topical sympathetic and parasympathetic agents, such as pilocarpine, atropine, tropicamide, and phenylephrine.

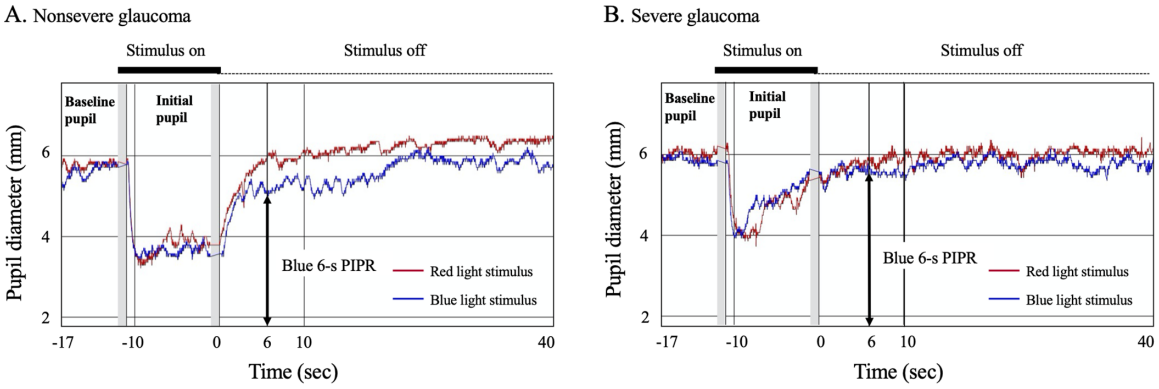

Figure 1. Time trace plots of the pupillary light reflex. The representative pupillary light reflex is shown for patients with (**A**) nonsevere glaucoma and (**B**) severe glaucoma. The *red* and *blue traces* indicate the pupil diameter following red and blue light stimuli, respectively. Two *vertical thick gray lines* indicate light stimulus onset (−10 seconds) and offset (0 seconds). The *vertical black double-headed arrow line* indicates the blue six-second post-illumination pupil response.

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Table 1. Basic, Systemic, and Ophthalmic Parameters by Glaucoma Severity

	Glaucoma Severity		
	Nonsevere	**Severe**	***P* Value**
Number of eyes	35	113	
Basic parameters			
Age, mean (SD), year	67.5 (14.3)	71.4 (10.2)	0.07
Sex, male (%)	13 (37.1)	57 (50.4)	0.17
Body mass index, (SD)	22.4 (3.2)	22.7 (3.5)	0.64
Systemic parameters			
Hypertension, number (%)	11 (31.4)	52 (46.0)	0.13
Diabetes, number (%)	8 (22.9)	23 (20.4)	0.75
Use of oral medication antihistamine and dopaminergic, number (%)	0 (0)	13 (11.5)	0.036
Ophthalmic parameters			
Cataract surgery, number (%)	6 (17.1)	50 (44.2)	0.004
Axial length, mean (SD), mm	24.6 (1.7)	24.3 (1.7)	0.38
Use of topical alpha$_2$-adrenergic receptor agonist, number (%)	3 (8.6)	11 (9.7)	0.84

SD, standard deviation.

Statistical Analyses

The unpaired *t*-test was used to analyze variables with a normal distribution, such as means ± standard deviations. The χ^2 test was used to compare categorical data. Mean differences in pupillary light reflex parameters according to the categorical glaucoma severity were assessed using analysis of covariance. We calculated the correlations between the PIPR parameters and continuous glaucoma severity using the Pearson's correlation coefficient. We used multivariable linear regression analyses to determine the association between PIPR parameters and continuous glaucoma severity. Table 1 summarizes the potential confounders according to basic parameters (age, sex, body mass index), clinical parameters (hypertension, diabetes, and use of oral medications antihistamines and dopaminergic), and ophthalmic parameters (cataract surgery, axial length, and use of topical alpha$_2$-adrenergic receptor agonist). All statistical analyses were performed using SPSS version 25 (IBM SPSS Statistics, Inc., Chicago, IL, USA), with two-sided *P* values < 0.05 indicating statistical significance.

Results

The mean ages of the 35 patients with nonsevere glaucoma and 113 patients with severe glaucoma were 67.5 ± 14.3 and 71.4 ± 10.2 years, respectively. No significant association was observed between glaucoma severity and basic parameters. The severe glaucoma group had a significantly higher prevalence of oral medication (antihistamine and dopaminergic) use and history of cataract surgery than the nonsevere glaucoma group ($P = 0.036$ and 0.004, respectively) (Table 1).

No significant difference between glaucoma severity and baseline pupil size before red and blue light stimulus was found. Initial pupil size during blue light stimulus in the severe glaucoma group was significantly larger than that in the nonsevere group (3.47 ± 0.66 vs. 3.82 ± 0.77 mm, $P = 0.015$) (Table 2). The association between continuous glaucoma severity and PIPR parameters are presented in Figure 2, Table 3, and Table 4. Simple linear regression analyses found that visual field MD was significantly inversely correlated with blue six-second PIPR amplitude ($P < 0.001$) and positively correlated with Net PIPR change ($P = 0.044$). Moreover, RNFL thickness was significantly inversely correlated with blue six-second PIPR amplitude ($P < 0.001$) and positively correlated with Net PIPR change and Net PIPR ($P = 0.004$ and $P = 0.012$, respectively). However, during red light stimulus, no significant correlation was found between visual field MD and red six-second PIPR amplitude (Fig. 2).

Table 2. Pupil-Related Parameters by Glaucoma Severity

	Glaucoma Severity		
	Nonsevere	**Severe**	***P* Value**
Baseline pupil size, mean (SD), mm			
red light	5.28 (1.19)	5.00 (1.06)	0.22
blue light	4.93 (1.15)	4.85 (0.99)	0.72
Initial pupil size, mean (SD), mm			
red light	3.92 (0.84)	4.12 (0.88)	0.26
blue light	3.47 (0.66)	3.82 (0.77)	0.015

SD, standard deviation.

Multivariable linear regression analyses adjusting for age, sex, body mass index, hypertension, diabetes, oral medications (antihistamines and dopaminergic) use, cataract surgery, axial length, and topical alpha$_2$-adrenergic receptor agonist use revealed that worsening in visual field MD was significantly associated with higher blue six-second PIPR amplitude (regression coefficient per −1 dB worsening, 0.25; 95% confidence intervals [CI], 0.14, 0.37; $P < 0.001$) (Table 3). Similarly, thinner RNFL thickness was significantly associated with higher blue six-second PIPR amplitude, lower Net PIPR change, and lower Net PIPR (blue six-second PIPR amplitude: regression coefficient per 10-µm thinning of RNFL thickness, 1.29; 95% CI, 0.72 to 1.87; $P < 0.001$; Net PIPR change: regression coefficient, −0.70; 95% CI, −1.26 to −0.14; $P = 0.015$; and Net PIPR: regression coefficient, −0.03; 95% CI, −0.05 to −0.001; $P = 0.044$) (Table 4).

In association between categorical glaucoma severity and PIPR parameters, during red light stimulus, no significant difference in red six-second PIPR amplitude was found between the nonsevere and severe glaucoma group ($P = 0.54$). During blue light stimulus, however, the severe glaucoma group had a significantly higher blue six-second PIPR amplitude than the nonsevere glaucoma group (93.5% ± 6.3% vs. 90.9% ± 6.2%; $P = 0.036$).

Axial length was inversely correlated with six-second PIPR amplitude ($P < 0.001$) in the univariable. In the multivariable linear regression analysis, we found a consistently significant association between axial length and six-second

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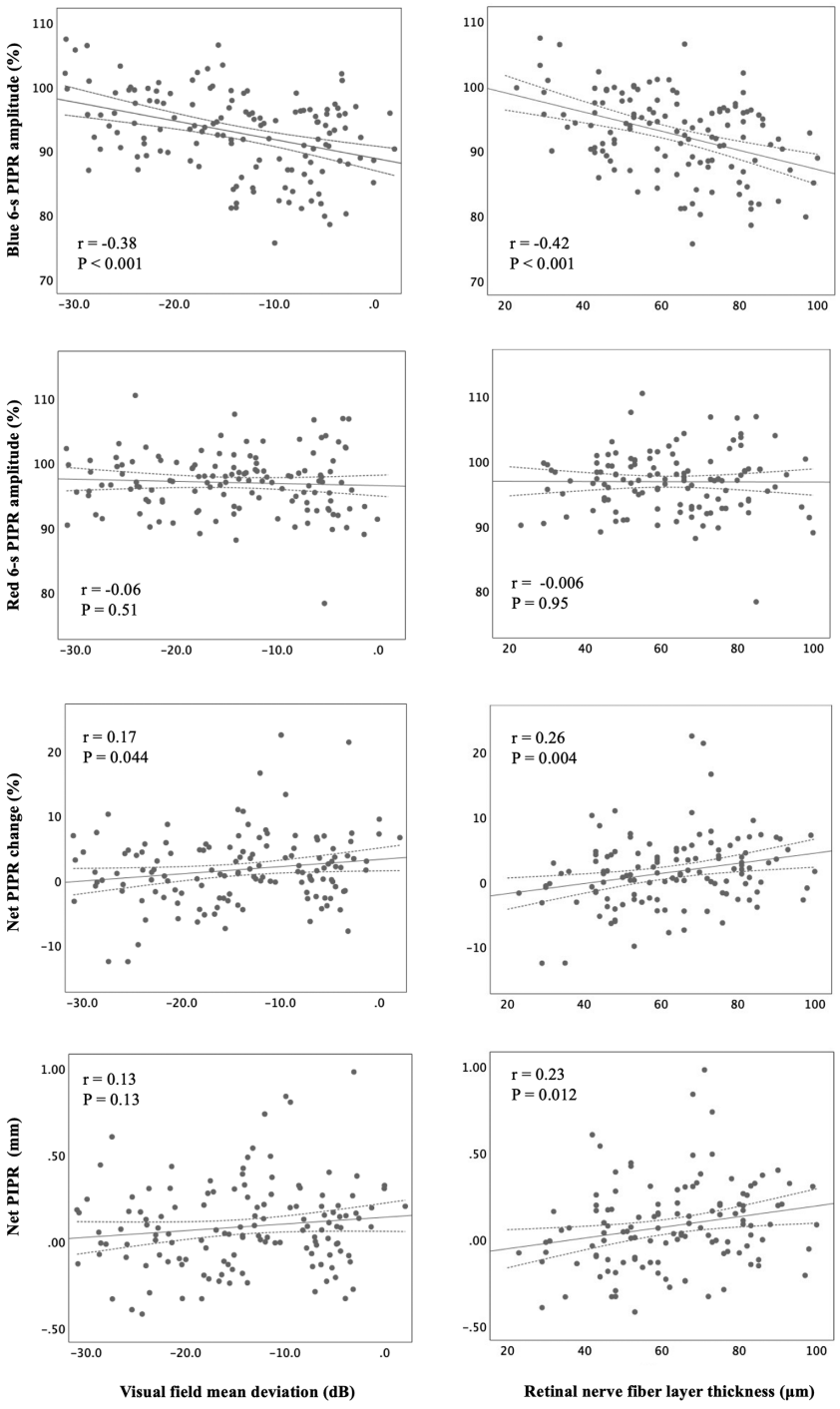

Figure 2. The scatter plots and correlation analyses between the post-illumination pupil response parameters and glaucoma severity. The *solid line* indicates regression lines, whereas the *dotted lines* indicated the 95% confidence intervals.

PIPR, adjusted for potential confounders including functional (regression coefficient, −0.86; $P = 0.006$; Table 3) and structural glaucoma severity (regression coefficient, −0.87; $P = 0.01$; Table 4). In addition, the topical alpha$_2$-adrenergic receptor agonist use was significantly associated with a higher six-second PIPR amplitude in the univariable ($P = 0.018$) and multivariable linear regression analyses (regression coefficient, 4.49; $P = 0.006$; Table 3 and regression coefficient, 4.43; $P = 0.008$; Table 4).

Subgroup analyses of patients with primary open-angle glaucoma (n = 117) showed that multivariable linear regression analyses adjusting for potential confounders indicated that worsening in visual field MD and thinner RNFL thickness were also significantly associated with higher blue six-second PIPR amplitude (regression coefficient per −1 dB worsening in visual field MD, 0.23; 95% CI, 0.09, 0.38; $P = 0.002$ and regression coefficient per 10 µm thinning of RNFL thickness, 1.29; 95% CI, 0.56 to 2.02; $P = 0.001$, respectively).

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Table 3. Multivariable Analysis for the Association Between Functional Glaucoma Severity and PIPR Parameters

	Adjusted Model											
	Blue 6-s PIPR Amplitude, %			Red 6-s PIPR Amplitude, %			Net PIPR Change, %			Net PIPR, mm		
Variables	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value
Visual field mean deviation (per 1 dB worsening)	0.25	0.14, 0.37	<0.001	0.03	−0.08, 0.16	0.63	−0.10	−0.21, 0.01	0.08	−0.003	−0.01, 0.002	0.23
Age (per year)	0.02	−0.08, 0.11	0.76	−0.07	−0.16, 0.01	0.08	−0.05	−0.14, 0.05	0.34	−0.003	−0.01, 0.001	0.17
Sex (male vs female)	−2.01	−3.99, −0.03	0.047	−1.41	−3.13, 0.31	0.11	0.06	−1.87, 1.98	0.96	−0.003	−0.09, 0.09	0.95
Body mass index (per BMI)	0.06	−0.23, 0.34	0.70	0.05	−0.20, 0.31	0.68	0.03	−0.26, 0.33	0.82	0.002	−0.01, 0.02	0.78
Hypertension (yes vs no)	0.77	−1.27, 2.82	0.46	−0.43	−2.15, 1.30	0.63	1.39	−0.61, 3.39	0.17	0.06	−0.03, 0.16	0.18
Diabetes (yes vs no)	−0.79	−3.21, 1.63	0.52	1.12	−0.94, 3.18	0.28	1.60	−0.74, 3.94	0.18	0.08	−0.03, 0.19	0.13
Oral medication (antihistamine and dopaminergic) use (yes vs no)	−1.07	−4.42, 2.28	0.53	−2.61	−5.32, 0.10	0.06	0.08	−3.06, 3.22	0.96	0.04	−0.11, 0.19	0.58
Cataract surgery (yes vs no)	2.58	0.50, 4.66	0.015	0.82	−0.92, 2.56	0.35	−0.90	−2.92, 1.12	0.38	−0.04	−0.13, 0.06	0.44
Axial length (per mm)	−0.86	−1.48, −0.25	0.006	−0.40	−0.92, 0.13	0.14	0.50	−0.10, 1.10	0.10	0.02	−0.01, 0.05	0.16
Topical alpha$_2$-adrenergic receptor agonist use (yes vs no)	4.49	1.32, 7.66	0.006	2.09	−0.44, 4.62	0.11	−2.62	−5.69, 0.45	0.09	−0.12	−0.26, 0.03	0.11

BMI, body mass index.

Adjusted for the potential confounders in Table 1 [age, sex, body mass index, hypertension, diabetes, use of oral medication (antihistamine and dopaminergic), cataract surgery, axial length, and topical alpha$_2$-adrenergic receptor agonist use]

Table 4. Multivariable Analysis For The Association Between Structural Glaucoma Severity And PIPR Parameters

	Adjusted Model											
	Blue 6-Second PIPR Amplitude, %			Red 6-Second PIPR Amplitude, %			Net PIPR Change, %			Net PIPR, mm		
Variables	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value	Regression Coefficient	95% CI	*P* Value
RNFL thickness (per 10-µm thinning)	1.29	0.72, 1.87	<0.001	0.01	−0.51, 0.52	0.98	−0.70	−1.26, −0.14	0.015	−0.03	−0.05, −0.001	0.044
Age (per year)	−0.01	−0.11, 0.09	0.80	−0.07	−0.16, 0.01	0.10	−0.03	−0.13, 0.07	0.57	−0.003	−0.01, 0.002	0.26
Sex (male vs female)	−1.84	−3.90, 0.23	0.08	−1.47	−3.32, 0.37	0.12	0.88	−1.15, 2.92	0.39	0.04	−0.05, 0.14	0.37
Body mass index (per BMI)	−0.07	−0.38, 0.24	0.65	0.06	−0.22, 0.35	0.67	0.12	−0.20, 0.44	0.45	0.004	−0.01, 0.02	0.64
Hypertension (yes vs no)	0.94	−1.15, 3.04	0.38	−0.36	−2.19, 1.47	0.70	1.17	−0.90, 3.24	0.27	0.05	−0.05, 0.15	0.30
Diabetes (yes vs no)	−0.64	−3.18, 1.91	0.62	0.98	−1.25, 3.21	0.39	0.81	−1.68, 3.30	0.52	0.05	−0.06, 0.17	0.38
Oral medication (antihistamine and dopaminergic) use (yes vs. no)	−1.75	−5.18, 1.68	0.31	−2.88	−5.73, −0.03	0.047	0.47	−2.75, 3.69	0.77	0.06	−0.09, 0.20	0.46
Cataract surgery (yes vs. no)	1.97	−0.23, 4.18	0.08	1.02	−0.91, 2.95	0.30	−0.68	−2.85, 1.49	0.54	−0.02	−0.12, 0.08	0.69
Axial length (per mm)	−0.87	−1.53, −0.21	0.01	−0.41	−0.99, 0.17	0.16	0.37	−0.29, 1.02	0.27	0.01	−0.02, 0.04	0.40
Topical alpha$_2$-adrenergic receptor agonist use (yes vs no)	4.43	1.15, 7.70	0.008	2.35	−0.35, 5.06	0.09	−2.26	−5.46, 0.95	0.17	−0.10	−0.25, 0.05	0.18

BMI, body mass index.

Adjusted for the potential confounders in Table 1 (age, sex, body mass index, hypertension, diabetes, oral medication [antihistamine and dopaminergic] use, cataract surgery, axial length, and topical alpha$_2$-adrenergic receptor agonist use).

Discussion

The current cross-sectional study involving 148 patients with glaucoma investigated the association between ipRGC function and glaucoma severity evaluated using visual field MD and RNFL thickness. Our study showed a significant association between impaired ipRGC function and functional and structural glaucoma severity independent of potential confounders, such as age, sex, body mass index, hypertension, diabetes, oral medication use (antihistamines and dopaminergic drugs) use, history of cataract surgery, axial length, and topical alpha$_2$-adrenergic receptor agonist use. Regarding the strengths of this study, we used multivariable analyses with a large sample size to adjust for the various potential confounders among patients with glaucoma.

The significant association between PIPR and glaucoma severity shown in our study was consistent with the findings of four previous clinical studies that revealed associations between impaired ipRGC function and glaucomatous damage, such as RNFL thinning and visual field defects.[14,16–18] Earlier studies have adjusted for some potential confounders, such as age,[14,16–18] sex,[18] and refractive error,[18] for comparisons between glaucoma and control groups but not between glaucoma cases. They also excluded participants with diabetes,[14,17] cataract surgery history,[16–18] and high myopia.[18] The present study included a large number of patients with glaucoma (n = 148) and confirmed this association using multivariable analysis adjusted for various known potential confounders. Consistent with our findings, the results of a histological study using human retina of donor eye showed a 75% decrease in ipRGC density in eyes with severe glaucoma.[9] However, an experimental study using the rodent models of experimental glaucoma showed that ipRGCs were resistant to N-methyl-D-aspartate-induced cell injury and intraocular pressure elevation.[27,28] This inconsistency may have been caused by the underestimation of the total number of ipRGCs in the animal model given that morphological studies in mice dependent on the immunostaining of melanopsin fail to detect all types of ipRGCs.[29]

The ipRGCs may potentially mediate the relationship between glaucoma and decreased melatonin secretion. Melatonin has been widely used as an indicator of circadian biological rhythm. Moreover, melatonin secretion from the pineal gland is regulated by the suprachiasmatic nuclei, which have ability to modulate circadian biological rhythm, through non–image-forming light from the ipRGCs in the retina.[30] An experimental study using a rodent chronic ocular hypertension model showed a 71.7% reduction in ipRGC axons to suprachiasmatic nuclei in glaucomatous rats and alteration in circadian locomotor activity.[31] Moreover, our earlier clinical study on 118 patients with glaucoma and 395 participants without glaucoma reported a decrease in melatonin secretion in the former. Furthermore, patients with functionally and structurally severe glaucoma had lower melatonin levels compared with those with mild glaucoma.[3] These results support the finding that impaired ipRGC function decreases melatonin secretion in patients with glaucoma.

The loss of ipRGC in patients with glaucoma affects entrainment of circadian biological rhythm in suprachiasmatic nuclei, possibly promoting clinical manifestations of circadian disruption, such as sleep disturbance, mood disorders, and diminished circadian blood pressure variability. Several studies have reported subjective sleep disturbances using self-report examination in patients with glaucoma.[32,33] Moreover, a cross-sectional study on 32 patients with glaucoma showed low objective sleep quality, such as sleep efficiency and total sleep time, evaluated through polysomnography in patients with glaucoma.[4] Meanwhile, our cross-sectional study of a community-based cohort found that participants with glaucomatous optic disc had a 2.5 times higher prevalence of depressive symptoms compared with those without the same.[6] Moreover, another report revealed higher nighttime blood pressure and diminished circadian blood pressure variability in patients with glaucoma.[7] Melatonin has been known to be involved in the aforementioned circadian rhythm disorders.[34–36] Consequently, glaucoma may induce sleep disturbance, mood disorders, and diminished circadian blood pressure variability through decreased melatonin secretion mediated by ipRGCs.

In PIPR, axial length and topical alpha$_2$-adrenergic receptor agonist use may be confounding factors in patients with glaucoma. First, axial length and PIPR showed a significant association in our study. In a recent clinical study involving 45 young adults (mean age, 24.1 years), blue light-stimulated pupils were more constricted and recovered slower in participants with greater hyperopia.[22] In contrast, another clinical study involving 59 healthy participants (mean age, 43.7 years) showed no effect of refractive error on PIPR.[37] Thus, the association between axial length and PIPR remains unclear. We were unable to accurately compare our findings with those of earlier studies owing to differences in age and the presence of glaucoma. Axial length and refractive error thus warrant further investigation. Second, topical alpha$_2$-adrenergic receptor agonist use is known to cause myosis[19] and potentially affects PIPR due to the decrease in light exposure to ipRGCs. Our results indicated a significant association between impaired ipRGC function and topical alpha$_2$-adrenergic receptor agonist use. However, two earlier studies reported that topical alpha$_2$-adrenergic receptor agonist use did not affect PIPR in patients with glaucoma.[16,18] These inconsistent results may be a result of differences in the sample size between our study (n = 148) and earlier work (n = 38 and 46).[16,18]

The current study has several limitations worth noting. First, given our cross-sectional study design, we could not clearly determine whether glaucoma progression affected ipRGC function. A prospective study is needed to determine whether glaucoma progression promotes the loss of ipRGCs. Second, cataract severity was not evaluated in the present study. Cataracts cause decreased light transmission to the retina, possibly facilitating circadian misalignment. A randomized clinical trial showed that cataract surgery influenced the circadian rhythm through melatonin secretion.[38] Thus, instead of assessing cataract severity, multivariable analysis was performed to adjust for cataract surgery.

In conclusion, the current study on 148 patients with glaucoma revealed that functional and structural glaucoma severity was significantly associated with impaired ipRGC function, even after adjusting for potential confounders. Our results indicate a possible circadian misalignment in patients with glaucoma through impaired ipRGC function.

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