

Glaucoma

Asymmetric Macular Structural Damage Is Associated With Relative Afferent Pupillary Defects in Patients With Glaucoma

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PURPOSE. We examined the relationship between relative afferent pupillary defects (RAPDs) and macular structural damage measured by macular thickness and macular ganglion cell-inner plexiform layer (mGCIPL) thickness in patients with glaucoma.

METHODS. A cross-sectional study was done of 106 glaucoma patients and 85 healthy individuals from the Diagnostic Innovations in Glaucoma Study. All subjects underwent standard automated perimetry (SAP) and optic nerve and macular imaging using Cirrus Spectral Domain Optical Coherence Tomography (SDOCT). Glaucoma was defined as repeatable abnormal SAP or progressive glaucomatous changes on stereo photographs. Pupil responses were assessed using an automated pupillometer, which records the magnitude of RAPD (RAPD score), with additional RAPD scores recorded for each of a series of colored stimuli (blue, red, green, and yellow). The relationship between RAPD score and intereye differences (right minus left eye) in circumpapillary retinal nerve fiber layer (cpRNFL) thickness, mGCIPL, macular thickness, and SAP mean deviation (MD), was examined using linear regression.

RESULTS. There was fair correlation between RAPD score and asymmetric macular structural damage measured by intereye difference in mGCIPL thickness ($R^2 = 0.285$, $P < 0.001$). The relationship between RAPD score and intereye difference in macular thickness was weaker ($R^2 = 0.167$, $P < 0.001$). Intereye difference in cpRNFL thickness ($R^2 = 0.350$, $P < 0.001$) and SAP MD ($R^2 = 0.594$, $P < 0.001$) had stronger association with RAPD scores compared to intereye difference in mGCIPL and macular thickness.

CONCLUSIONS. Objective assessment of pupillary responses using a pupillometer was associated with asymmetric macular structural damage in patients with glaucoma.

Keywords: pupillary response, pupillary reflex, glaucoma, macula, structural damage

Glaucoma is an optic neuropathy characterized by progressive loss of retinal ganglion cells (RGCs) with associated structural changes to the optic nerve head and retinal nerve fiber layer (RNFL).¹ Although glaucoma usually is bilateral, asymmetry is common with reported intereye differences in structural and functional measurements, including differences in circumpapillary RNFL (cpRNFL) thickness² and standard automated perimetry (SAP) sensitivity.³⁻⁵ As intereye differences in cpRNFL thickness and SAP sensitivity are uncommon in healthy individuals,⁶ detection of asymmetry may be useful for identifying glaucoma.

The pupillary light reflex can be used as an indicator of the integrity of the afferent input from retina and optic nerve. A relative afferent pupillary defect (RAPD) is present when there is asymmetry in the pupillary light reflex and is indicative of asymmetric impairment of the anterior afferent visual pathways.^{7,8} The pupillary light reflex can be assessed using the swinging flashlight test,⁹ or using automated pupillometry, which allows quantification of the pupillary response.^{7,10}

Although RAPDs are observed in patients with glaucoma and other optic neuropathies,^{11,12} little is known about the degree of asymmetric structural damage that must be present before an RAPD becomes apparent. Previous studies have examined intereye differences in SAP, cpRNFL,^{8,13,14} and combined information from SAP and RNFL.¹⁵ However, to the best of our knowledge, none has examined the effect of macular damage on the pupillary light reflex in glaucoma. The macula, which is the portion of the posterior retina between the vascular arcades containing xanthophyll,^{16,17} is likely to contribute significantly to the pupillary light response as it contains more than 50% of RGCs.¹⁸ The macula is an attractive region for identifying structural damage due to its importance for central vision and as it may be subject to less anatomic variability than circumpapillary structures.^{19,20} Using spectral-domain optical coherence tomography (SDOCT), it is possible to obtain objective measurements of macular structures, such as the ganglion cell-inner plexiform layer (mGCIPL), which contains RGC bodies that contribute to the afferent pathway of the pupillary reflex.



TABLE 1. Baseline Demographic and Clinical Characteristics of Study Patients (Mean \pm SD)

	Healthy Subjects, N = 85 Subjects	Glaucoma Patients, N = 106 Subjects	P Value
Age,† y	57.3 \pm 14.6	70.1 \pm 10.3	<0.001
Sex, female, n (%)*	48 (57%)	48 (45%)	0.146
Ethnicity, n (%)*			0.152
Caucasian	62 (73%)	64 (60%)	
African-American	13 (15%)	26 (25%)	
Other	10 (12%)	16 (15%)	
SAP MD worse eye,† dB	0.0 \pm 0.8	-6.9 \pm 7.3	<0.001
SAP MD better eye,† dB	0.6 \pm 0.8	-2.9 \pm 4.4	<0.001
SAP MD intereye difference,† dB	0.6 \pm 0.4	4.0 \pm 4.9	<0.001
cpRNFL thickness worse eye,† μ m	90.7 \pm 10.8	68.3 \pm 12.0	<0.001
cpRNFL thickness better eye,† μ m	94.5 \pm 9.9	77.5 \pm 11.6	<0.001
cpRNFL thickness intereye difference,† μ m	3.8 \pm 3.6	9.2 \pm 7.6	<0.001
mGCIPL thickness worse eye,† μ m	80.8 \pm 6.6	65.2 \pm 10.7	<0.001
mGCIPL thickness better eye,† μ m	82.5 \pm 6.6	71.9 \pm 8.0	<0.001
mGCIPL thickness intereye difference,† μ m	1.7 \pm 1.8	6.8 \pm 6.8	<0.001
Macular thickness worse eye,† μ m	262.1 \pm 24.0	251.1 \pm 19.4	<0.001
Macular thickness better eye,† μ m	266.9 \pm 25.1	260.3 \pm 19.4	0.046
Macular thickness intereye difference,† μ m	5.2 \pm 5.0	9.3 \pm 8.7	<0.001

* Fisher's exact test.

† *t*-test.

The purpose of the current study was to determine the relationship between intereye differences in mGCIPL thickness and magnitude of RAPD measured using automated pupillometry in patients with glaucoma. We aimed to determine the degree of asymmetry in macular structural damage that might be required for a RAPD to become detectable.

METHODS

Study Sample

This was a cross-sectional study including both eyes of 191 participants from the Diagnostic Innovations in Glaucoma Study (DIGS), a previously described prospective longitudinal study.²¹ The study was conducted at the Hamilton Glaucoma Center of the Department of Ophthalmology, University of California San Diego (UCSD). Written informed consent was obtained from all participants, and the institutional review board and human subjects committee at the University of California San Diego prospectively approved all methods. All study methods adhered to the tenets of the Declaration of Helsinki for research involving human subjects and the study was conducted in accordance with the regulations of the Health Insurance Portability and Accountability Act.

At each visit subjects underwent comprehensive ophthalmologic examination, including best-corrected visual acuity, slit-lamp biomicroscopy, IOP measurement, gonioscopy, dilated funduscopy examination, simultaneous stereoscopic optic disc photography (Kowa WX3D; Kowa Optimed, Inc., Tor-

rance, CA, USA), and SAP using the Swedish interactive threshold algorithm (SITA Standard 24-2). Only subjects with open angles on gonioscopy were included. Subjects were excluded if they had a best-corrected visual acuity less than 20/40 or any other ocular or systemic disease that could affect the optic nerve or the visual field. Patients using systemic or topical cholinergic or anticholinergic medications (including pilocarpine) that might affect pupil responses were excluded.

Participants were classified as healthy or glaucomatous according to worse eye diagnosis. Eyes were classified as glaucomatous if they had repeatable (≥ 3 consecutive) abnormal SAP test results on the 24-2 Humphrey visual field analyzer test (Carl Zeiss Meditec, Inc., Dublin, CA, USA). An abnormal SAP result was defined by a pattern standard deviation outside the 95% confidence limits (CI) or a glaucoma hemifield test result outside the reference range. Healthy subjects were recruited from the general population and required IOP ≤ 21 mm Hg with no history of increased IOP and normal SAP in both eyes. Each subject had Cirrus SDOCT (software v. 5.2, model 4000; Carl Zeiss Meditec, Inc.), SAP, and automated pupillometry within a 4-month interval.

Optical Coherence Tomography

The Cirrus SDOCT was used to acquire cpRNFL, mGCIPL, and total macular thickness measurements. This device has been described in detail previously.²² The cpRNFL thickness measurements were acquired using the optic disc cube 200 \times 200 protocol using a 3-dimensional scan of a 6 \times 6-mm area

TABLE 2. Pupillary Reflex Characteristics of Healthy Subjects Compared to Glaucoma Patients (Mean \pm SD)

	Healthy Subjects, N = 85 Subjects	Glaucoma Patients, N = 106 Subjects	P Value
Response latency intereye difference, ms	4.54 \pm 3.69	6.95 \pm 6.72	0.031
Peak Constriction intereye difference, ms	0.13 \pm 0.13	0.20 \pm 0.21	0.006
Absolute RAPD score	0.15 \pm 0.10	0.30 \pm 0.35	0.006
Absolute RAPD score - blue	0.23 \pm 0.21	0.41 \pm 0.46	0.013
Absolute RAPD score - green	0.20 \pm 0.17	0.42 \pm 0.39	<0.001
Absolute RAPD score - yellow	0.19 \pm 0.16	0.39 \pm 0.40	<0.001
Absolute RAPD score - red	0.27 \pm 0.26	0.50 \pm 0.49	<0.001

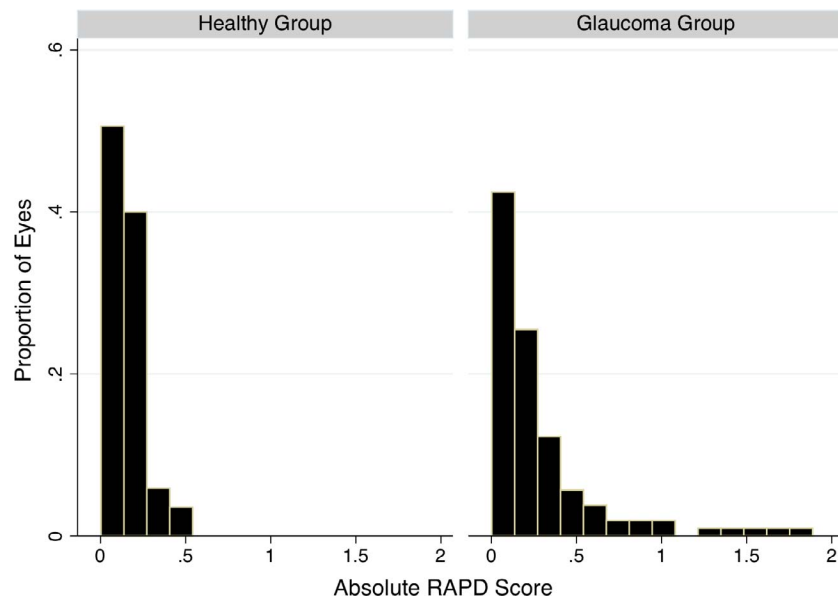


FIGURE 1. Histogram showing the distribution of absolute RAPD scores in healthy eyes and those with glaucoma in at least one eye.

centered on the optic disc. Average cpRNFL thickness was calculated automatically from a 3.46-mm diameter circular scan (10.87 mm length) around the optic disc. The macular cube 200 × 200 protocol was used to acquire macular thickness data. This protocol is based on a 3-dimensional scan centered on the macula in which information from a 1024 (depth) × 200 × 200 point parallelepiped is collected. The ganglion cell analysis algorithm automatically segmented the mGCIPL, defined by the outer boundary of RNFL and outer boundary of inner plexiform layer.^{23,24} Cirrus SDOCT images were reviewed and included if well centered, signal strength > 7 and movement artifacts and segmentation errors were absent.

Standard Automated Perimetry

All visual fields were evaluated by the UCSD Visual Field Assessment Center (VisFACT).²⁵ Visual fields with more than 33% fixation losses or more than 15% false-positive errors were excluded. Visual fields were reviewed further for the following artifacts: eyelid and rim artifacts, fatigue effects, inappropriate fixation, evidence that the visual field results were caused by a disease other than glaucoma and inattention.

Pupillometry Stimuli

Pupil responses were studied using the RAPDx (Konan Medical USA, Inc., Irvine, CA, USA), a binocular infrared pupillometer.^{11,15} The device measures bilateral pupil responses to monocularly presented visual stimuli. Stimuli are created using a single LCD screen with a central physical barrier creating two

optical channels. The screen displays a target for fixation and during testing each portion of the screen can be enabled selectively to achieve separate stimulation of each eye. The screen is viewed at infinity through a pair of 50-mm objective lenses providing an approximate 25° field of view in each eye. Eyes also are illuminated by a pair of infrared emitting diodes, with peak emission at 880 nm, mounted at a 35° angle.

Under infrared conditions information regarding the “dark” pupil diameter is captured as camera pixels and this measurement is converted to millimeters using a scaling factor. The stimulus then is presented as a series of trials, either to the full field of each eye or limited to predetermined regions. The size, color, intensity, and length of time of each stimulus were controlled automatically via proprietary software.

Procedure

The full field stimulus testing strategy was used in this study. This stimulus extends to approximately 18° from fixation. Each trial consisted of a period of stimulation followed by a period of darkness during which the cameras record continuously. The total time of each trial was 2.0 seconds plus a 100-ms post-trial rest period during which no images were acquired. The full field white stimulus was presented for 200 ms of the 2.1-second duty cycle and 18 trials (nine for each eye) used this stimulus for a total test time of 37.8 seconds. The right eye was stimulated first, followed by the left, then the right, with continued stimulation alternating between eyes. The full-field stimuli had luminances of 384 (white stimulus), 88 (red stimulus; 605 nm), 27 (green stimulus; 555 nm), 23 (blue stimulus; 440 nm), and 380 (yellow stimulus; 576 nm) cd/m², and during all tests, there was a nominal background

TABLE 3. Results of Univariable Regression Analysis of RAPD Score Investigating the Relationship Between Intereye Difference (Δ, Right Minus Left Eye) in cpRNFL Thickness, mGCIPL Thickness, Macular Thickness, and MD From SAP

Intereye Difference, Δ	Constant	Coefficient	R ²	P Value
cpRNFL thickness, μm	0.001	0.022	0.350	<0.001
mGCIPL thickness, μm	0.023	0.026	0.285	<0.001
Macular thickness, μm	0.022	0.141	0.167	<0.001
SAP MD, dB	0.018	0.059	0.594	<0.001

TABLE 4. Results of Multivariable Regression Analysis of Absolute RAPD Score and Absolute Intereye Difference (Δ, Right Minus Left Eye) in mGCIPL Thickness, Including Average MD From SAP as a Covariable

Intereye Difference, Δ	Coefficient	95% CI	P Value
mGCIPL thickness, μm	0.014	0.01 to 0.02	<0.001
Average SAP MD, dB	-0.021	-0.03 to -0.01	<0.001
Constant	0.114	0.07 to 0.16	<0.001

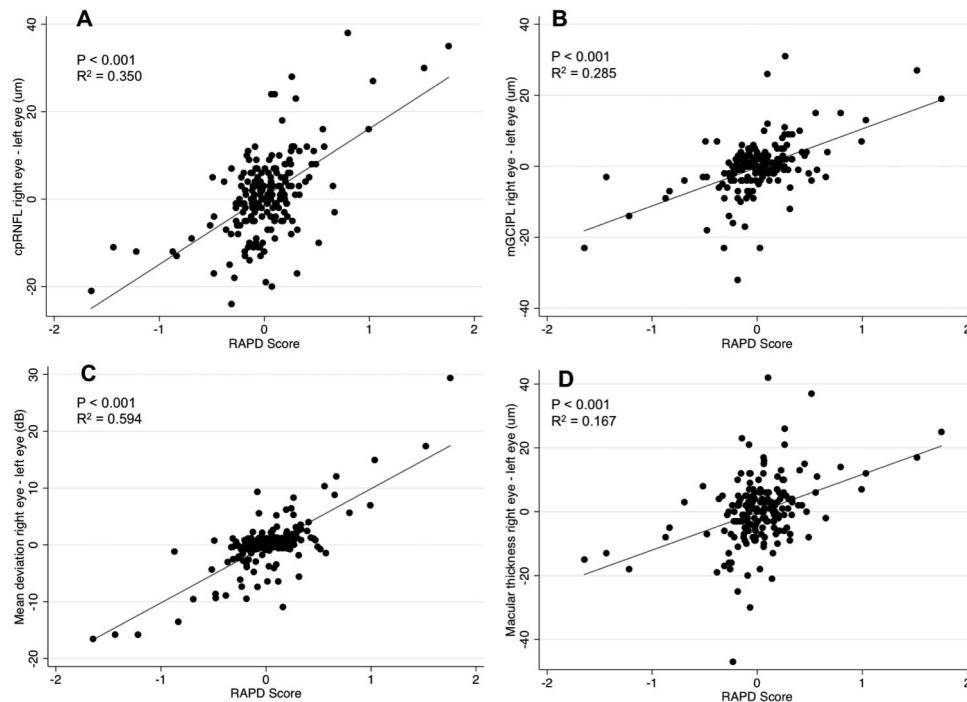


FIGURE 2. Scatter plot showing RAPD score association with intereye differences in cpRNFL thickness (A), mGCIPL thickness (B), macular thickness (C), and SAP MD (D). All intereye differences were calculated subtracting left eye values from right eye values. The ordinary least squares regression lines are shown.

luminance of 0.01 cd/m². Testing was conducted under dark room conditions with an illuminance of <0.5 lux.

The pupillometer includes proprietary analysis software, which was used to parse the generated pupil diameter waveforms into specific metrics. The repetitions from each eye were averaged (median) before analysis to minimize noise inherent in the pupil responses. Parameters measured by the pupillometer include prestimulus pupil diameter (in millimeters), minimum pupil diameter following the stimulus (in millimeters), response amplitude (i.e., maximal contraction of the pupil as a percentage of the prestimulation size, that is, the prestimulus pupil diameter minus the minimum pupil diameter, divided by the prestimulus pupil diameter), response latency (time in milliseconds between stimulus onset and time when pupil velocity has reached 50% of the peak velocity of constriction), and time to peak constriction (in milliseconds). An RAPD is defined as a difference in average pupillary constriction (response amplitude) when each eye is stimulated monocularly.^{9,26} An index of the direction and magnitude of pupil response asymmetry, known as the RAPD score, is generated automatically by the RAPDx device. The RAPD score is calculated as the difference in the amplitude of pupil constriction between stimulation of the two eyes using the following formula:²⁷

$$RAPD\ score = 10 \times \log_{10}\left(\frac{od}{os}\right),$$

where *od* is the mean response amplitude in both eyes, in response to right eye stimulation, and *os* is the mean response amplitude in both eyes in response to left eye stimulation. An RAPD score of 0 would be expected for a healthy subject. A positive value indicates a relative abnormality of the left afferent system and a negative value indicates a relative abnormality of the right afferent system.²⁷ The RAPD score is useful as it confers information regarding the direction as well as the magnitude of an RAPD. However, to investigate the effect of potential confounders, such as average disease severity, the absolute RAPD score also was calculated as an overall measure of asymmetry of the afferent visual pathways, regardless of which eye was affected. The arithmetic difference in response amplitude on stimulation of the better eye and on stimulation of the worse eye also was calculated.

Statistical Analysis

Descriptive statistics included mean and standard deviation (SD) and *t*-tests for normally distributed variables; and median, interquartile range, and Wilcoxon rank-sum for nonparametrically distributed variables. The relationship between RAPD

TABLE 5. Results of Univariable Regression Analysis of RAPD Score in Different Colors Compared to Intereye Difference (Δ, Right Minus Left Eye) in mGCIPL, Macular, cpRNFL Thickness, and MD From SAP

Intereye Difference, Δ	Blue Color		Red Color		Green Color		Yellow Color	
	R ²	P Value	R ²	P Value	R ²	P Value	R ²	P Value
cpRNFL thickness, μm	0.235	<0.001	0.241	<0.001	0.286	<0.001	0.296	<0.001
mGCIPL thickness, μm	0.196	<0.001	0.211	<0.001	0.214	<0.001	0.232	<0.001
Macular thickness, μm	0.114	<0.001	0.064	<0.001	0.076	<0.001	0.118	<0.001
SAP MD, dB	0.417	<0.001	0.396	<0.001	0.478	<0.001	0.513	<0.001

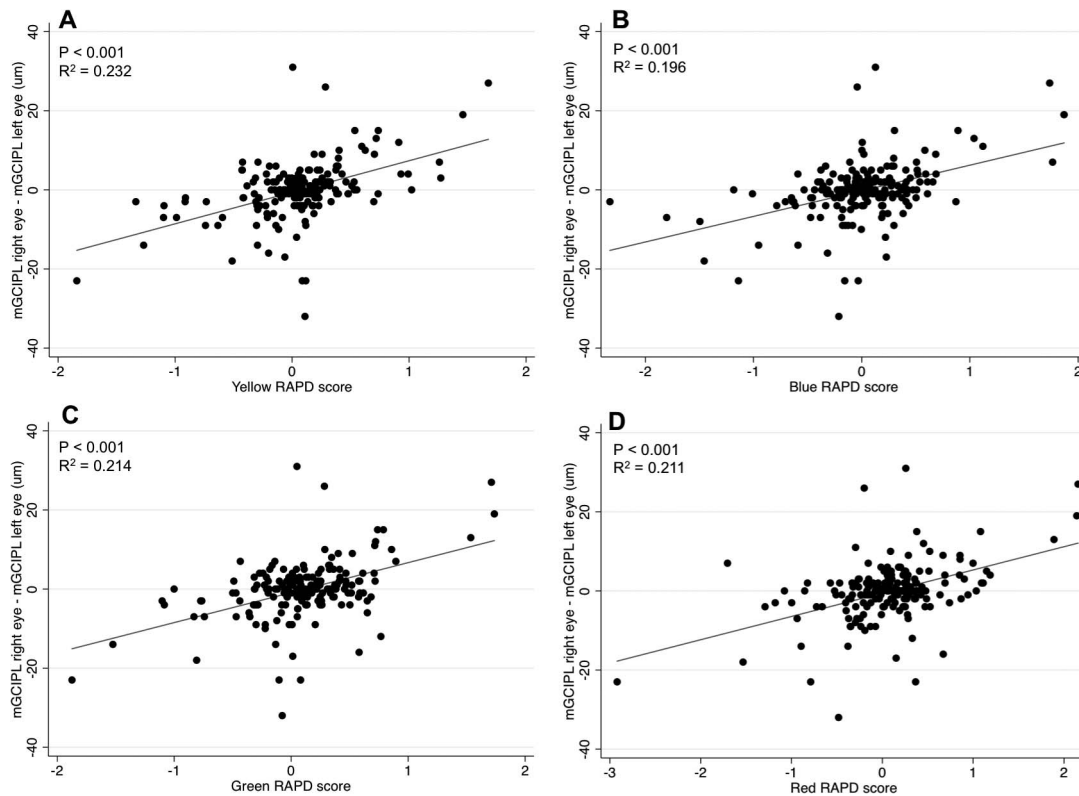


FIGURE 3. Scatter plot showing intereye difference of mGCIPL thickness association with different colors for RAPD score: *red* (A), *blue* (B), *green* (C), and *yellow* (MD) (D). All intereye differences were calculated subtracting left eye values from right eye values. The ordinary least squares regression lines are shown.

scores and intereye differences (right eye minus left eye) in structural parameters was examined using scatter plots and linear regression. The average cpRNFL thickness, mGCIPL, macular thickness, and SAP mean deviation (MD) were included in the analyses to evaluate the effect of disease severity on asymmetry needed for an RAPD. The effect of age also was examined as a covariate. All statistical analyses were performed with commercially available software (STATA, version 13; StataCorp LP, College Station, TX, USA). The α level (type I error) was set at 0.05.

RESULTS

The study included 106 patients with glaucoma and 85 healthy controls. Of those with glaucoma, 67 had glaucoma in both eyes, 33 had glaucoma in one eye and suspected glaucoma in the other, and 6 had glaucoma in one eye and a putative healthy fellow eye. The demographic and clinical characteristics of participants are summarized in Table 1.

Subjects with glaucoma had an average MD of -6.9 ± 7.3 dB in the worse eye and -2.9 ± 4.4 dB in the better eye, which was significantly worse than in healthy subjects ($P < 0.001$ for both comparisons). The cpRNFL, mGCIPL, and macular thickness also were significantly thinner in the worse and better eyes of those with glaucoma compared to controls (Table 1).

Those with glaucoma also had greater asymmetry in SAP and SDOCT measurements between eyes compared to healthy subjects. The mean (\pm SD) intereye difference in MD was 4.0 ± 4.9 dB in those with glaucoma compared to only 0.6 ± 0.4 dB in healthy subjects. Intereye difference in cpRNFL thickness was 9.2 ± 7.6 μ m in patients with glaucoma, compared to only 3.8 ± 3.6 μ m in healthy participants. Corresponding intereye

differences in mGCIPL thickness were 6.8 ± 6.8 and 1.7 ± 1.8 μ m, respectively.

The absolute RAPD score averaged 0.30 ± 0.35 in those with glaucoma compared to 0.15 ± 0.10 in healthy subjects ($P = 0.006$; Table 2). Figure 1 shows the distribution of absolute RAPD scores in healthy and glaucomatous subjects. Only 12 (14%) healthy subjects had an absolute RAPD score higher than 0.25 and none had higher than 0.50. However, in the glaucomatous group, 42 of 106 subjects (40%) had an absolute RAPD score higher than 0.25, 31 of 106 (29%) had an absolute RAPD score higher than 0.30, and 22 (21%) had an absolute RAPD score higher than 0.50. Subjects with glaucoma also had significantly greater intereye differences in pupil response latency and time to peak pupil constriction compared to healthy subjects ($P = 0.031$ and $P = 0.006$, respectively). Those with glaucoma also had, on average, a greater absolute RAPD score for blue ($P = 0.013$), green ($P < 0.001$), yellow ($P < 0.001$), and red ($P < 0.001$) color stimuli compared to healthy subjects (Table 2).

There was a significant association between RAPD scores and intereye differences in macular structural damage, as measured by intereye difference in mGCIPL thickness ($R^2 = 0.285$, $P < 0.001$). The relationship between RAPD score and intereye difference in macular thickness was weaker ($R^2 = 0.167$, $P < 0.001$) than that with mGCIPL. However, intereye differences in cpRNFL thickness ($R^2 = 0.350$, $P < 0.001$) and SAP MD ($R^2 = 0.594$, $P < 0.001$) had stronger associations with RAPD score than macular parameters (Table 3, Fig. 2).

Intereye asymmetry of glaucoma damage may be influenced by disease severity; for example, patients with bilateral end-stage glaucoma are likely to have less asymmetry than those with early glaucoma due to a floor effect in structural and functional measurements. In contrast, those with moderate glaucoma may have greater asymmetry than those with very early disease. Therefore, we included average MD of right and left eyes in a

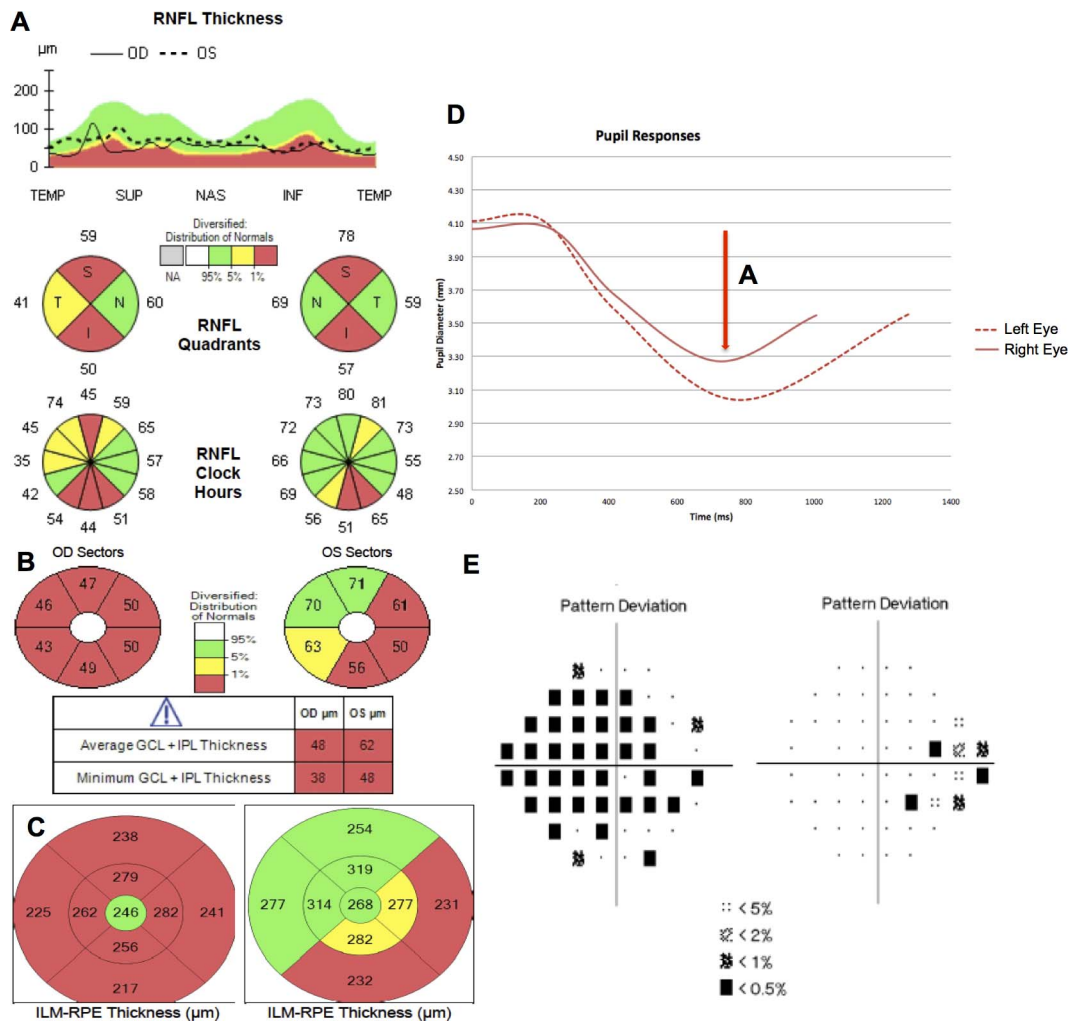


FIGURE 4. Example of a 77-year-old patient included in the study. Optical coherence tomography measurements of cpRNFL (A), mGCIPL (B), and total macular thickness (C) are shown. Average cpRNFL thickness was 48 and 62 μm in the right and left eyes. Automated pupillometry showing the average pupil response on right and left eye stimulation (D) revealed reduced response amplitude (denoted by the arrow and letter A) on stimulation of the right eye. Average response amplitude on right eye stimulation was -0.20 compared to 0.27 on left eye stimulation, giving an RAPD score of $10^{\log_{10}(0.20/0.27)} = -1.21$, indicating a relative abnormality of the right afferent pathway. Standard automated perimetry MD in the right and left eyes was -18.97 and -3.17 dB, respectively (E).

multivariable regression analysis. Greater intereye difference in mGCIPL thickness remained associated with greater absolute RAPD score (Table 4) but worse average MD also was associated with greater absolute RAPD scores ($P < 0.001$). RAPD score increased by 0.021 for each 1 dB worse average MD. Similar effects of disease severity also were noted on the association between RAPD score with asymmetries in the other structural and functional variables, that is, intereye cpRNFL thickness ($P < 0.001$); absolute intereye SAP MD difference ($P < 0.001$), and absolute intereye macular thickness difference ($P < 0.001$). In multivariable analyses, age had no significant influence on the relationship between absolute RAPD score and absolute intereye mGCIPL thickness difference ($P = 0.560$), intereye differences in cpRNFL thickness, macular thickness, or SAP MD ($P = 0.378$, $P = 0.156$, and $P = 0.888$, respectively).

Although the association between RAPD scores obtained using colored stimuli and intereye differences in structural and functional parameters were significant, the associations with RAPD score to the white stimulus generally were stronger (Table 5, Fig. 3). Examples of patients included in the study are shown in Figures 4 to 6. Figure 4 is an example of a patient with advanced glaucoma in the right eye and mild glaucoma in

the left eye showing a RAPD score of -1.21 , while Figure 5 shows a patient with advanced glaucoma in both eyes and a RAPD score of only -0.12 . Figure 6 shows a patient with mild glaucoma in both eyes and a RAPD score of only -0.09 .

DISCUSSION

This study has shown that the magnitude of RAPD measured using an automated pupillometer is correlated with measures of macular structural asymmetry in glaucoma. Subjects with larger RAPDs had greater intereye differences in macular thickness and mGCIPL thickness, in addition to greater intereye differences in cpRNFL and SAP MD. To our knowledge, this is the first study to evaluate the relationship between magnitude of RAPD and macular structural damage in glaucoma.

Patients with glaucoma were more likely to have intereye asymmetry in structural and functional measurements than healthy controls, likely because, although glaucoma typically is a bilateral disease, changes often are worse in one eye than the other. Patients with glaucoma also had larger RAPD scores than controls, with average RAPD scores of 0.30 ± 0.35 and 0.15 ± 0.10 , respectively. A significant association was seen between

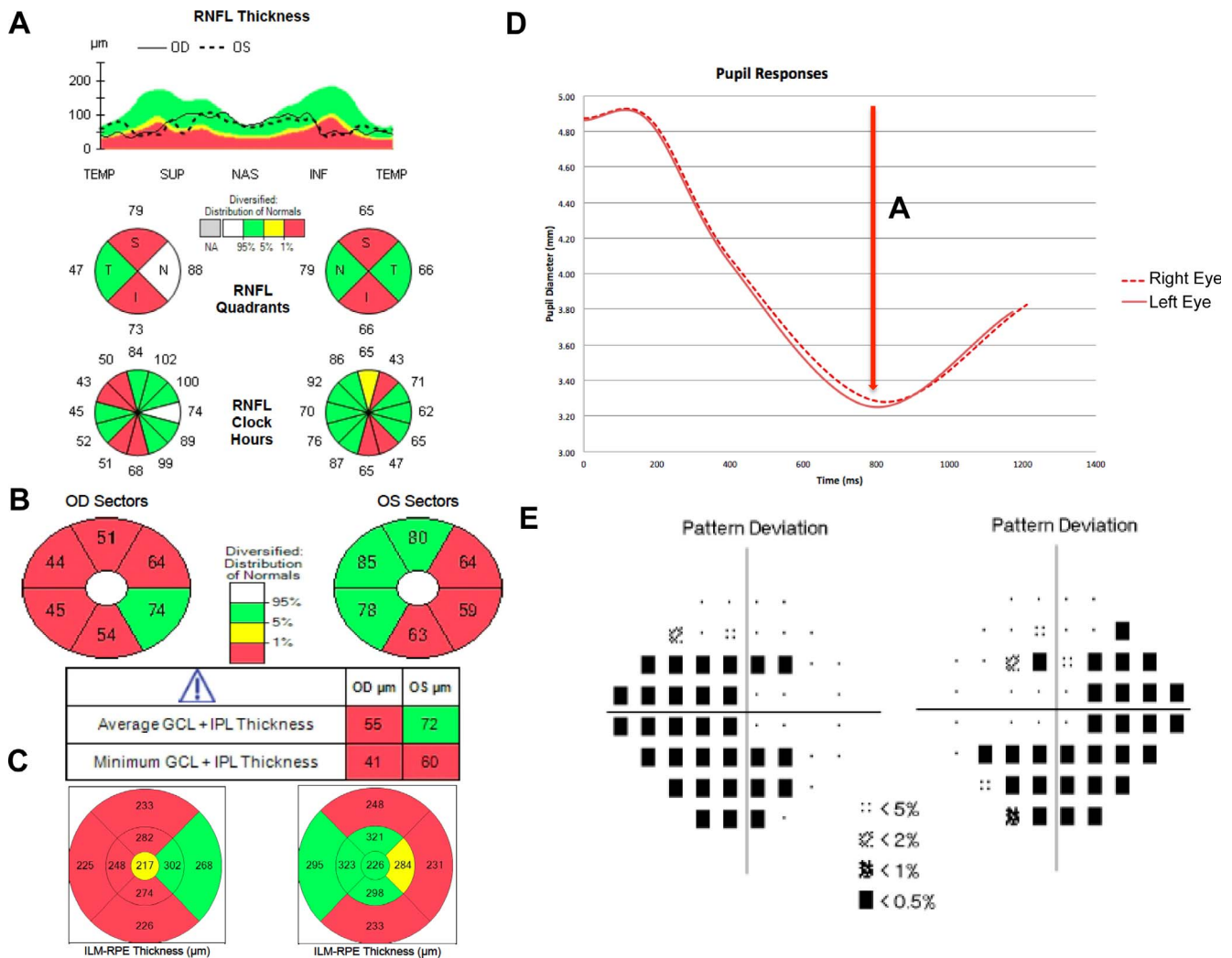


FIGURE 5. Example of 60-year-old patient with advanced glaucoma in both eyes. Optical coherence tomography measurements of cpRNFL (A), mGCIPL (B), and total macular thickness (C) are shown. Average cpRNFL thickness was 71 and 69 µm in the right and left eyes. Automated pupillometry showing the average pupil response on right and left eye stimulation (D) revealed reduced response amplitude (denoted by the arrow and letter A) on stimulation of the right eye. The average response amplitude on right eye stimulation was -0.33 compared to 0.34 on left eye stimulation, giving an RAPD score of $10 \cdot \log_{10}(0.33/0.34) = -0.12$, indicating a small relative abnormality of the right afferent pathway. Standard automated perimetry MD in the right and left eyes was -21.36 and 16.6 dB, respectively (E).

RAPD and intereye difference in mGCIPL ($R^2 = 0.285$; $P < 0.001$). For each 10-µm increase in mGCIPL asymmetry there was a 0.26 increase in RAPD score. Such association was stronger than that for total macular thickness, indicating that the mGCIPL parameter was a more useful indicator of the degree of pupillary defect in glaucoma. The stronger association between RAPD score and mGCIPL asymmetry is likely to be due to total macular thickness including nonneuronal support tissues, not directly involved in the pupillary light response. In contrast, mGCIPL thickness is a measurement of the inner layers containing RGCs.^{28–31}

Although asymmetries in macular measurements were associated with RAPD, we found that the relationships between RAPD score and asymmetry in cpRNFL and SAP MD were stronger still, with R^2 of 0.350 and 0.594, respectively. This is likely explained by the fact that although the macula accounts for a large proportion of RGCs, RGCs outside this area are likely also to contribute to the pupillary reflex. As cpRNFL thickness measurements and SAP MD depend on the integrity of all RGC axons throughout the retina, this probably explains why these parameters showed higher association with pupillometry results.

We found an RAPD score of 0.50 is likely to correspond to intereye differences in cpRNFL and SAP MD of approximately

23 µm and 8 dB, respectively, which are similar results to those reported previously using the swinging flashlight test, suggesting that an intereye difference in cpRNFL thickness of 17% to 27%, or an intereye difference in SAP MD of 9.5 to 12 dB, would correlate with an RAPD of 0.3 to 0.6.^{8,14} As one might expect, we also found that the relationship between magnitude of RAPD and intereye difference in mGCIPL thickness depends on disease severity, with worse average MD associated with greater absolute RAPD scores. Our study included patients with a range of disease severities; however, there were few with very severe disease. Only 19 patients had a MD in the worse of eye of worse than -15 dB. It is important to note that in patients with advanced but symmetrical glaucoma in both eyes there is not likely to be an RAPD. Therefore, worse average MD will not always be associated with a larger RAPD.

Although the swinging flashlight test is a simple and widely available test of RAPD, automated pupillometry is likely to be a more sensitive method of quantifying abnormalities of the pupillary light reflex. Lankaranian et al.⁹ examined the ability of pupillometry to detect glaucoma and found an RAPD in 56% in 70 patients with glaucoma, compared to only 29% using swinging flashlight test. A recent systematic review, including

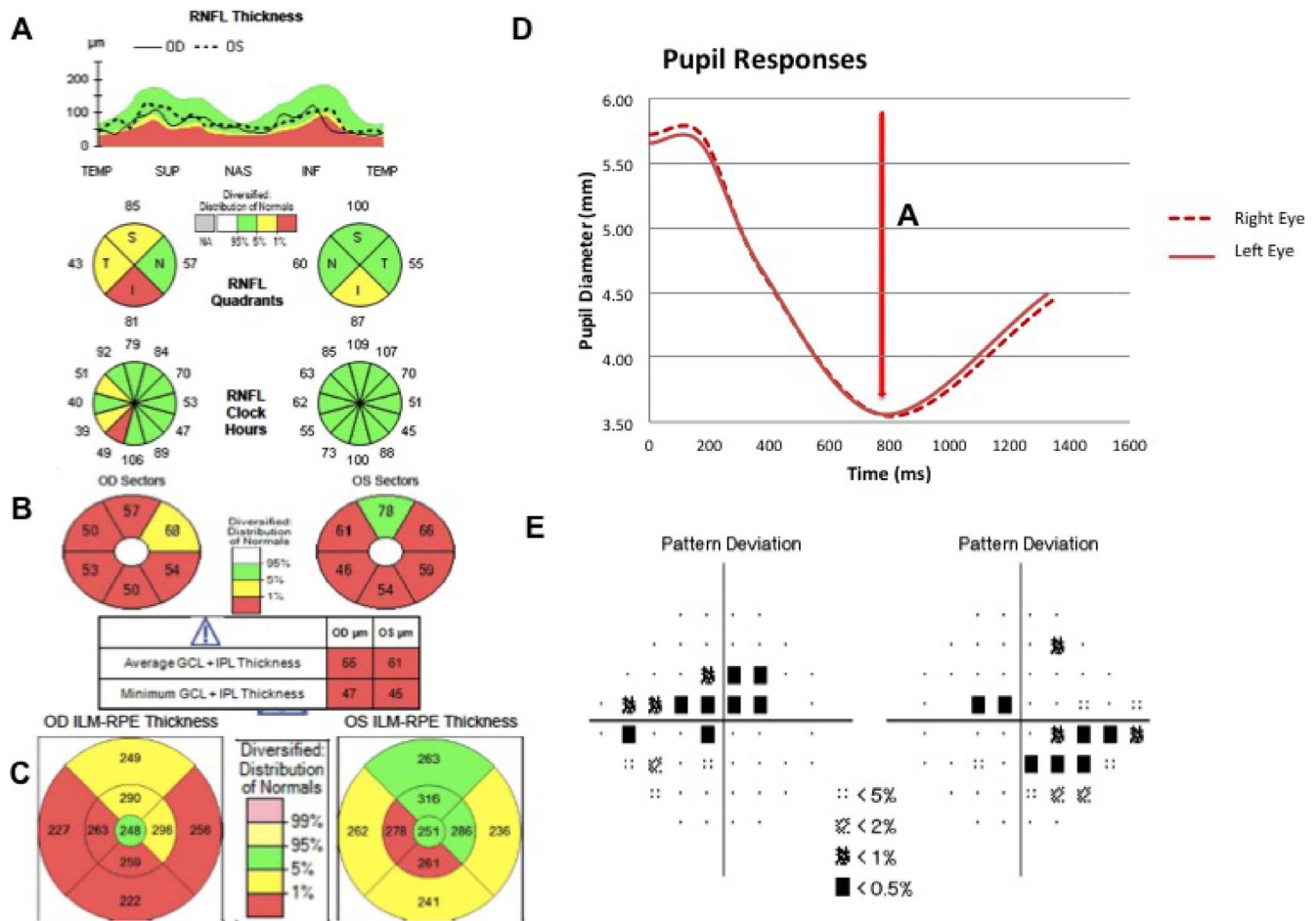


FIGURE 6. Example of 66-year-old patient with mild glaucoma in both eyes. Optical coherence tomography measurements of cpRNFL (A), mGCIPL (B), and total macular thickness (C) are shown. Average cpRNFL thickness was 68 and 69 μm in the right and left eyes. Automated pupillometry showing the average pupil response on right and left eye stimulation (D) revealed reduced response amplitude (denoted by the arrow and letter A) on stimulation of the right eye. The average response amplitude on right eye stimulation was -0.32 compared to 0.33 on left eye stimulation, giving an RAPD score of $10^{\log_{10}}(0.32/0.33) = -0.09$, indicating a small relative abnormality of the right afferent pathway. Standard automated perimetry MD in the right and left eyes was -3.97 and -3.76 dB, respectively (E).

12 studies of pupillometry, concluded that automated pupillometers were able to detect smaller intereye differences in pupil reactions than detectable using the swinging flashlight test.¹³ Further, although the swinging flashlight test is quick and easy to perform, it requires considerable practice to perform reliably and can be challenging in patients with dark irides or small or poorly reactive pupils.^{26,32} We did not use the swinging flashlight test in our study; however, previous studies have suggested an RAPD of more than 0.3 is likely to be abnormal.³² In the present study, 29% of patients with glaucoma had an RAPD score of 0.3 or more.

We evaluated pupil responses to a range of colored stimuli but the RAPD score to the white stimulus had the strongest association with all structural and functional parameters. However, patients with glaucoma did have greater RAPD scores for blue, green, yellow, and red colored stimuli compared to healthy subjects and there was an association between RAPD scores obtained using colored stimuli, and asymmetric structural and functional damage. These results are in agreement with previous studies that showed RAPD scores using different wavelength stimuli were greater in glaucoma patients and correlated with intereye SAP MD difference.^{33,34}

The study had limitations. The magnitude of RAPD can be influenced by factors other than glaucoma, such as brightness, location of the stimulus or time between stimuli.³⁵ However, to

minimize these effects, only the large full field flash stimulus was used. As another limitation of our study, there was a significant difference in the average age between glaucoma and controls. However, we did not find any significant influence of age on the magnitude of the RAPD score. In addition, although the pupillometer used different colored stimuli, it is important to emphasize that these stimuli do not selectively target specific RGC subtypes, such as intrinsically photosensitive RGCs (ipRGCs).³⁶ This would be an interesting topic for further study, since targeting specific subgroups of RGCs may allow earlier detection of damage.

In conclusion, we found a significant association between the magnitude of an RAPD and intereye difference in mGCIPL and macular thickness. However, RNFL thickness parameters and the degree of visual field loss asymmetry had stronger relationships with pupillometry-measured RAPD and may be better indicators of the degree of expected pupillary afferent defect.

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