Original Article

Correlation of iris collagen and *in-vivo* anterior segment structures in patients in different stages of chronic primary angle-closure in both eyes

Chuchen Chung[1], Miaomiao Dai[1,2], Jialiu Lin[1], Zhonghao Wang[1], Hailiu Chen[1], Jingjing Huang[1]

Purpose: To compare the density of iris collagen and anterior segment parameters in eyes with chronic primary angle closure glaucoma (CPACG) and their fellow eyes with confirmed or suspected primary angle closure (PAC/PACS). **Methods:** Nineteen patients with CPACG in one eye and PAC/PACS in the fellow eye requiring trabeculectomy in the CPACG eye and iridectomy in the fellow eye were recruited. Anterior segment optical coherence tomography (AS-OCT) measurements were conducted under light and dark conditions. Iris specimens, obtained by iridectomy/trabeculectomy, were analyzed by sirius red polarization for quantifying type I/III collagen density. AS-OCT parameters and type I/III collagen densities were compared between the two eyes. **Results:** The iris curvatures were flatter in CPACG eyes in light and dark conditions ($P < 0.05$). The iris areas in light condition and iris thicknesses in dark condition were smaller in CPACG eyes ($P < 0.05$). The density of collagen type I in CPACG eyes was lower ($P = 0.048$). The light-to-dark changes in CPACG eyes and PAC/PACS eyes, respectively, were −0.679 ± 0.701 and −1.627 ± 0.802 mm for pupil diameters, and 0.069 ± 0.113 and 0.258 ± 0.157 mm^2 for iris areas, which differed significantly ($P < 0.001$). With the decrease of type I collagen, anterior chamber width increased and iris areas decreased in dark condition. **Conclusion:** Dynamic changes in pupil diameter and iris areas differed significantly between CPACG eyes and their fellow PAC/PACS eyes. Decreased type I collagen density in iris tissue was associated with decreased iris area and increased anterior chamber width, which may contribute to disease progression.

Access this article online

Website: www.ijo.in

DOI: 10.4103/ijo.IJO_1406_18

Quick Response Code:

Key words: Anterior segment optical coherence tomography, iris collagen, primary angle closure

Primary angle-closure glaucoma (PACG) is a major cause of vision loss, especially in Asia.[1,2] Many previous studies have found that shallow anterior chamber depth (ACD), short axial length (AL), increased lens thickness (LT), and greater lens vault (LV) are risk factors for PACG.[3-5] In addition, altered dynamic factors of the iris have also been suggested as risk factors.[6] The International Society of Geographical and Epidemiological Ophthalmology (ISGEO) classification for primary angle-closure (PAC) include primary angle closure suspect (PACS), PAC, and PACG,[7] which can be regarded as a progressive spectrum of the disease. However, factors contributing to disease progression are not well understood. In clinical practice, we have seen many patients with significant asymmetry in disease stage between the two eyes. Our previous studies found that a thin and anteriorly bowed iris is related to PACG development.[8] In addition, dynamic changes in iris parameters with light-to-dark transition differed significantly among CPACG eyes, fellow PAC/PACS eyes, and normal eyes.[9]

Despite the enormous amount of published data on biometric measurements of PACG, there has been little data on the iris collagen in PACG eyes.[10,11] Previous reports have speculated that differences in iris collagen content may in part explain the difference seen in dynamic responses of angle closure.[10] However, there has been no study correlating iris collagen with *in vivo* measurements. In the current study, we investigate the relationship between iris collagen and *in vivo* anterior segment parameters in patients with advanced stage PACG in one eye and PAC or PACS in the fellow eye, in order to elucidate whether they contribute to disease development.

Methods

This is a cross-sectional comparative study. Consecutive patients diagnosed by two glaucoma specialists (JH & ZW) with advanced CPACG in one eye and PAC or PACS in the fellow eye and in whom trabeculectomy in the CPACG eye and iridectomy in the PAC/PACS eye was clinically indicated in order to prevent the high risk eyes from PAC/PACS to PACG according to the consensus of diagnosis and treatment of primary glaucoma by the Chinese Glaucoma Society and previous studies[12-15] were recruited. The study was conducted at the Department of Glaucoma in Zhongshan Ophthalmic Center of Sun Yat-sen University (Guangzhou,

[1]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, [2]Department of Ophthalmology, Shunde Hospital of Southern Medical University (The First People's Hospital of Shunde), Foshan, China

Correspondence to: Dr. Jingjing Huang, State Key Laboratory of Ophthalmology, Department of Glaucoma, Zhongshan Ophthalmic Center, Sun Yat-sen University, 7 Jinsui Road, Guangzhou - 510623, China. E-mail: hjjing@mail.sysu.edu.cn

Manuscript received: 21.08.18; Revision accepted: 28.04.19

Cite this article as: Chung C, Dai M, Lin J, Wang Z, Chen H, Huang J. Correlation of iris collagen and *in-vivo* anterior segment structures in patients in different stages of chronic primary angle-closure in both eyes. Indian J Ophthalmol 2019;67:1638-44.

China) from December 2014 to March 2015. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. Informed consents were obtained from all patients. All subjects underwent detailed ocular examinations including best-corrected visual measurement, slit-lamp examination, stereoscopic optic disc examination with a 90-diopter lens, and intraocular pressure (IOP) measurement by Goldmann applanation tonometry. Gonioscopy was performed in the dark using a Goldmann one-mirror lens at high magnification. The extent of peripheral anterior synechiae (PAS) in each eye was assessed by both glaucoma specialists (JH & ZW). If a discrepancy existed, a second examination by the two glaucoma specialists was performed and the results averaged. Visual field examination was performed with the Humphery perimetry (SITA standard 30-2 or 24-2, Carl Zeiss, Dublin, CA, USA). Axial length measurement was performed by A-scan ultrasonic biometry (Model KN-3000A; Quantel Co, Ltd., France).

CPACG, PAC, PACS were defined using the ISGEO classification.[7] PACS occurs when greater than 270° of the posterior trabecular meshwork is not visible on gonioscopy. PAC is PACS associated with PAS and/or elevated IOP without glaucomatous damage to the optic disc. CPACG is PAC with evidence of optic nerve damage. PAC and CPACG eyes must exhibit no signs of acute angle closure attack such as "glaukomflecken" or iris sphincter palsy. The diagnosis of glaucomatous damage was based on (1) glaucomatous optic neuropathy, defined as a cup-to-disc (C/D) ratio >0.6 or asymmetry of > 0.2 between the two eyes; (2) loss or thinning of the neuroretinal rim or retinal nerve fiber layer on clinical examination; (3) reliable and repeatable glaucomatous visual field defect, defined in a SITA standard 30-2 or 24-2 Humphery perimetry as two or more contiguous points with a pattern deviation $P < 0.01$ sensitivity loss or more, or three or more contiguous points with $P < 0.05$ sensitivity loss or more, in the superior or inferior arcuate areas (compared with that in perimeter-defined age-matched control subjects), or a 10-dB difference across the nasal horizontal midline at two or more adjacent locations and an abnormal result in a glaucoma hemifield test (false-positive/negative rate <15%, fixation loss < 10%).

The exclusion criteria were: (1) secondary angle closure such as neovascular, uveitic, traumatic glaucoma, and so on; (2) previous laser or intraocular surgery performed on either eyes; (3) patients with subluxated lens or intumescent cataract; (4) patients with uveal effusion or retinal detachment; (5) patients with a history or current use of topical or systemic cholinergic agents within 7 days that could affect the iris or pupil size;[8,9,16] (6) patients with axial length less than 19 mm in either eyes.

AS-OCT (Visante; Carl Zeiss Meditec, Inc., Dublin, CA, USA.) examinations and measurements were performed by the same trained physician (JL) who was masked to the clinical data. Intraocular pressures of the examined patients were controlled below 30 mmHg in both eyes using anti-glaucoma eye drops when indicated. This is to minimize corneal edema, which may affect AS-OCT measurements of the anterior segment. Topical cholinergic agents were discontinued for at least 7 days prior to AS-OCT examinations. No prostaglandin analogs or osmotic agents were used as they may affect anterior segment measurements. AS-OCT examinations were first performed with the patients sitting in a bright room with strengthened lighting (illumination 750 to 800 Lux, Model TES-1339; TES Electrical Corp.) and then in a dark room (illumination 0.2 to 1 Lux). Subjects adapted to the dark for at least 5 min prior to examination. During AS-OCT scanning, an internal fixation target was used with the subjects' refractive correction to perform the measurements in non-accommodative conditions. The anterior segment was scanned perpendicularly centered over the pupil, using the horizontal standard anterior segment single-scan mode (0° to 180°), for a total of three times. A central corneal reflex was recommended for good repeatability. The best image was selected and stored in the machine for data collection.

Using the special caliper in the instrument, AS-OCT linear parameters such as the anterior chamber depth (ACD), anterior chamber width (ACW), pupil diameter (PD), angle opening distance at 500 μm from the scleral spur (AOD500 μm), iris thickness at 500 μm, 750 μm, 1,000 μm from the iris root (IT500 μm, IT750 μm, IT1000 μm), iris curvature (IC), lens vault (LV), lens thickness (LT) were measured as described previously.[3,17] All area measurements were performed using the "Magnetic Lasso Tool" in Photoshop as described in our previous studies.[9,18] The anterior chamber area (ACA) is the cross-sectional area of the anterior chamber bordered by the posterior surface of the cornea, the anterior surface of the iris, and the anterior surface of the lens within the pupil. The angle recess area (ARA750 μm) is the enclosed triangular area demarcated by the anterior iris surface, trabecular meshwork, and corneal endothelium out to a distance of 750 μm from the scleral spur. Iris area (I-area) is the cross-sectional area of the iris [Figs. 1 and 2]. Every parameter was measured 3 times and averaged. For anterior chamber angle and iris parameters, both nasal and temporal aspects were measured and then averaged.

One day after AS-OCT examinations, iris tissue specimens were obtained during surgical iridectomy/trabeculectomy in both eyes by the same surgeon (JH). Surgical iridectomy and trabeculectomy were performed as described previously.[19] For iridectomy, a 2.5 mm long incision was made in the cornea just anterior to the limbus and at the anterior edge of the limbal corneal vessels. Pressure was applied on the scleral side of the incision in an attempt to prolapse the iris into the incision. Then, a peripheral iridectomy was performed ensuring both the stromal and the pigment layers were held. After the return of the iris to anterior chamber, one 10-0 nylon suture was placed across the center of the corneal incision. For trabeculectomy, briefly, conjunctive flap and scleral flap were made, then sclerostomy was performed after paracentesis. Peripheral iridectomy was performed by grasping the peripheral iris through the sclerostomy with a fine-toothed forceps. Closure of the scleral flap and suturing the conjunctival flap were followed. Both incisions of iridectomy and trabeculectomy were made in the superior quadrants between 11 and 1 o'clock.

Each iris specimen obtained was fixed in 10% formaldehyde and embedded in paraffin. All specimens were cut in 6 μm sections for sirius red (Leagene Biotechnology, Beijing, China) staining. Polarization microscopy (ZEISS axioplan2 imaging, Carl Zeiss Microscopy, LLC, NY, USA) was used to differentiate type I and type III collagen fibers and to

quantitate their proportions. Type I collagen fibers were recognized by its thick fibers and bright red or yellow staining, while type III collagen fibers were characterized by slander fibers and green staining. Images under the polarization microscopy were saved by ZEISS Axiocam system and AxioVision software (Carl Zeiss Microscopy, LLC, NY, USA). The quantitative measurements of type I and type III collagen fibers were performed similarly to a previous study.[10] Briefly, 3 randomly fields were selected and photographed in each slide at a magnification of 20 × 10. Quantitative measurement was performed with ImageJ Image Processing software (ImageJ 1.50g, National Institutes of Health, Bethesda, USA) by the same researcher (CC) who was masked to the clinical data. Type I and type III collagen fibers were differentiated by "split channels" in ImageJ software due to their different colors. The researcher manually drew the borderline of the stained iris tissue [Fig. 3]. The morphometry of the cumulative areas of type I collagen, type III collagen, and total area of iris tissue were measured automatically by the software. The average of the ratios of cross-sectional area of type I and type III collagen fibers divided by total area of iris tissue in the 3 randomly selected photographs was used to represent the proportion of each type of collagen.

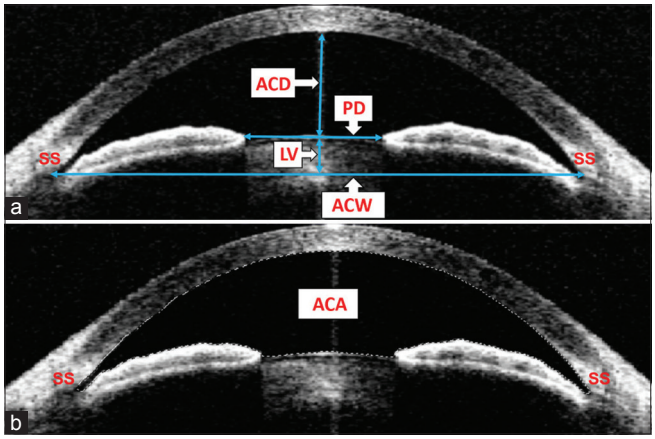

Figure 1: Determinations of ACD, ACW, LV, PD (a) and ACA (b) by AS-OCT. SS: scleral spur

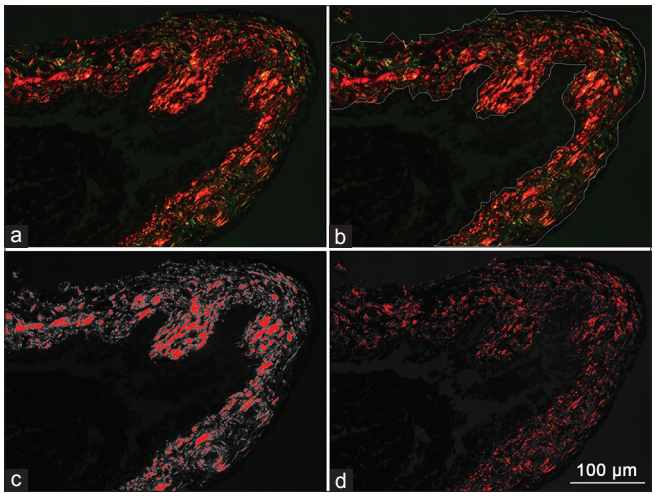

Figure 3: Quantitative measurement of type I/type III collagen fibers by ImageJ. (a) Type I collagen fibers are recognized by their thick fibers and bright red or yellow staining, type III collagen fibers are characterized by their slender fibers and green staining. (b) The researcher manually drew the borders of the stained iris tissue. The total area of demarcated iris tissue is measured. (c and d) Type I and type III collagen fibers are differentiated by "split channels" in ImageJ due to their different colors. The morphometry of the cumulative areas of type I/type III collagen are measured

Statistical analysis

Statistical analyses were performed using SPSS software version 18.0 (SPSS, Inc., Chicago, IL). The means and standard deviations of the anterior segment parameters and their change from light to dark, as well as collagen ratios were calculated. Differences between the CPACG eyes and their fellow eyes were examined using the paired *t* test.

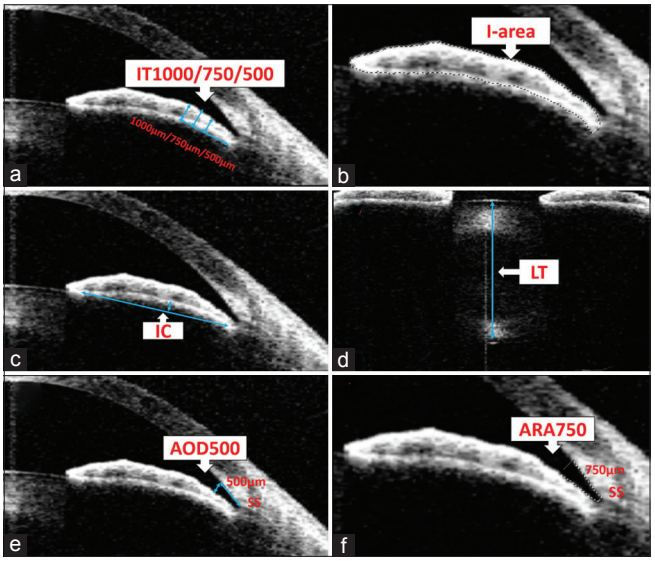

Figure 2: Determinations of IT500μm/750μm/1000μm (a), I-area (b), IC (c), LT (d), AOD500μm (e), and ARA750μm (f) by AS-OCT. SS: scleral spur

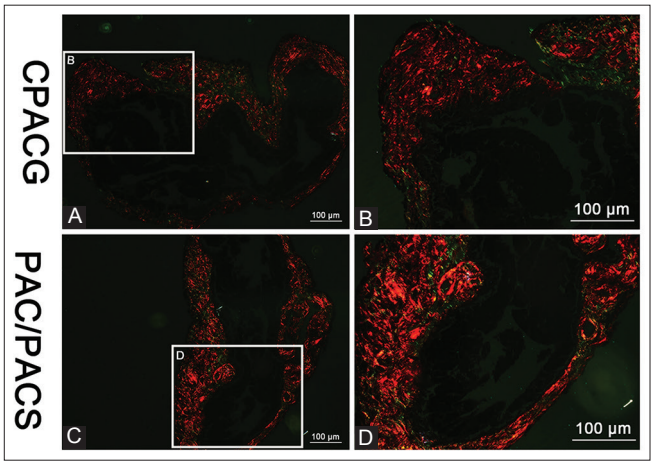

Figure 4: Iris tissue staining in one patient with CPACG in one eye and PAC/PACS in the fellow eye under polarization microscopy. Iris tissue stained by sirius red under polarization microscopy in one patient with one CPACG eye (A and B) and the other PAC/PACS eye (C and D) were shown. Collagen type I of iris tissue were thick and closely arranged express in bright red or yellow color, while collagen type III were slender and loose meshed fibers express in green color. Original magnifications of objective lens: (A, C)×10; (B, D)×20

Pearson correlation coefficient was used to assess the relationship between the anterior segment parameters and collagen ratios. $P < 0.05$ was considered significant.

Results

A total of 19 Chinese patients (8 male and 11 female) met the eligibility criteria and were recruited. The mean age was 60.26 ± 7.64 (45--75) years. There were 11 right eyes and 8 left eyes with CPACG. Because this was an intra-patient comparison study, 19 eyes were performed trabeculectomy, and 19 fellow eyes were performed iridectomy.

The clinical data of the CPACG eyes and their fellow PAC/PACS eyes are shown in Table 1. IOPs were much higher and PAS was more extensive in CPACG eyes than in PAC/PACS eyes ($P < 0.001$). PAC/PACS eyes had smaller C/D ratios and fewer visual field abnormalities than CPACG eyes ($P < 0.001$). There was no difference in axial lengths between CPACG eyes and their fellow eyes ($P = 0.358$).

In terms of anterior chamber and lens parameters [Table 2] in CPACG vs. its fellow eye, the ACDs were smaller in CPACG eyes in both light and dark conditions ($P = 0.012$ and 0.025, respectively). The ARAs in light condition, ACAs in dark condition were smaller in CPACG eyes ($P = 0.046$ and 0.027, respectively). There were no differences in ACW, AOD, LV, and LT in different lighting conditions ($P > 0.05$). Comparisons of iris measurements are shown in Table 3. Iris curvatures were smaller in CPACG eyes than in their fellow eyes in both light and dark conditions ($P = 0.009$ and 0.048, respectively). The iris areas in light condition and the iris thicknesses (IT750 μm and IT1000 μm) in dark condition were smaller in CPACG eyes ($P = 0.001$, 0.006 and 0.034, respectively). Pupil diameters were larger in light and smaller in dark among CPACG eyes ($P = 0.014$ and 0.019, respectively). The light-to-dark changes of pupil diameters, iris areas, and ACAs were smaller in CPACG eyes ($P < 0.001$, 0.001, 0.001, respectively) [Table 4].

Under polarization microscopy, collagen type I of iris tissue were thick and closely arranged express in bright red or yellow color, while collagen type III were slender and loose meshed fibers express in green color [Fig. 4]. Compared with those in PAC/PACS eyes, type I collagen in CPACG irises were looser and had a lower proportion [Fig. 4]. The quantitative measurements of the two types of collagen are shown in Table 5. The density of type I collagen in CPACG irises was lower ($P = 0.048$), but there was no difference in type III collagen density ($P = 0.374$).

Correlations between type I collagen measurements and anterior segment parameters under light and dark conditions are shown in Table 6. Decreased type I collagen density was associated with increased ACW under dark condition, and decreased AOD, ARA under light condition, as well as decreased I-area in dark condition ($P < 0.05$) [Table 6 and Fig. 5]. There were no correlations between type I and type III collagen measurements and light-to-dark changes of anterior-segment parameters ($P > 0.05$), nor between type III collagen measurements and anterior segment parameters under light and dark conditions ($P > 0.05$).

Discussion

The role of the iris in the pathogenesis of PAC is gaining more scrutiny recently.[20,21] In our previous studies[8,9] and the current study, thinner iris, smaller iris area and curvature, and less light-to-dark changes in pupil diameter and iris area were found in PACG eyes than their fellow PAC/PACS eyes. Type I and type III collagen fibers are the two major types in the iris stroma.[22] Although studies have investigated the expression of type I and type III collagen fibers in the iris of PACG eyes,[10] this is the first to assess the correlations between collagen

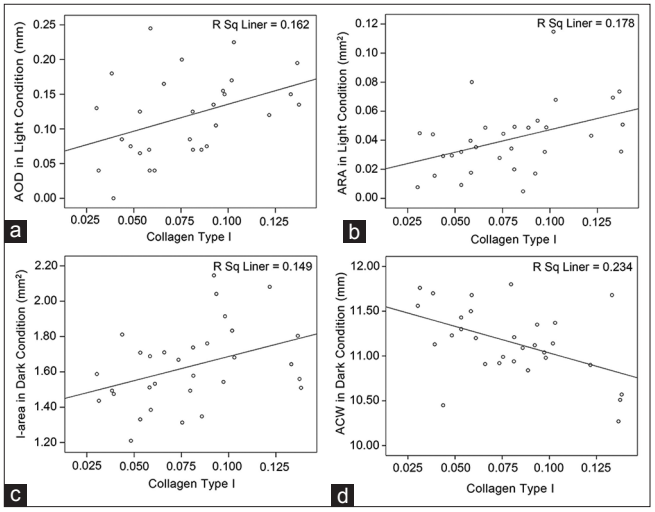

Figure 5: Scatter plots of AOD (a), ARA (b), I-area (c), and ACW (d) versus collagen measurements (type I). Decreased type I collagen density is correlated with decreased AOD and ARA under light condition, increased ACW and decreased I-area under dark condition. The solid line represents the best-fit line. R^2: Coefficient of determination; ACW: anterior chamber width; ARA: angle recess area; AOD: angle opening distance; I-area: iris area

Table 1: Clinical data of recruited eyes

	CPACG eyes (*n*=19)	Fellow PAC/PACS eyes (*n*=19)	*P*
IOP on examination (mmHg)	29.35±16.64	14.60±6.12	<0.001
Extent of PAS (clock-hours)	9.24±2.49	1.68±2.16	<0.001
C/D ratio	0.8±0.23	0.4±0.16	<0.001
MD of VF (dB)	-21.308±9.059	-4.401±2.476	<0.001
PSD of VF (dB)	7.431±2.995	2.645±1.902	0.004
AL (mm)	22.40±0.744	22.36±0.683	0.358

P=*P* of paired *t* test between CPACG eyes and their fellow PAC/PACS eyes; CPACG=Chronic primary angle closure glaucoma; PAC=Primary angle closure; PACS=Primary angle closure suspect; IOP=Intraocular pressure; PAS=Peripheral anterior synechiae; C/D=Cup to disc; MD=Mean deviation; VF=Visual field; PSD=Pattern standard deviation; AL=Axial length

Table 2: Anterior chamber and lens measurements of recruited eyes under light and dark conditions

	CPACG eyes (*n*=19)	Fellow PAC/PACS eyes (*n*=19)	*P*
Light Condition			
ACW (mm)	11.104±0.473	11.149±0.465	0.726
ACD (mm)	1.917±0.220	2.017±0.236	0.012
ACA (mm²)	12.635±2.136	12.983±1.909	0.460
AOD (mm)	0.103±0.087	0.135±0.066	0.221
ARA (mm²)	0.032±0.028	0.049±0.028	0.046
LV (mm)	0.727±0.277	0.709±0.236	0.566
LT (mm)	4.790±0.342	4.734±0.310	0.161
Dark Condition			
ACW (mm)	11.169±0.347	11.135±0.452	0.278
ACD (mm)	1.928±0.220	2.021±0.229	0.025
ACA (mm²)	12.908±2.102	13.850±1.929	0.027
AOD (mm)	0.074±0.091	0.084±0.078	0.564
ARA (mm²)	0.026±0.033	0.034±0.025	0.217
LV (mm)	0.734±0.240	0.693±0.247	0.490
LT (mm)	4.776±0.318	4.782±0.318	0.816

P=*P* of paired *t*-test between CPACG eyes and their fellow PAC/PACS eyes; CPACG=Chronic primary angle closure glaucoma; PAC=Primary angle closure; PACS=Primary angle closure suspect; ACW=Anterior chamber width; ACD=Anterior chamber depth; ACA=Anterior chamber cross-sectional area; AOD=Angle opening distance 500 µm from the scleral spur; ARA=Angle recess area 750 µm from the scleral spur; LV=Lens vault; LT=Lens thickness

Table 3: Iris measurements of recruited eyes under light and dark conditions

	CPACG eyes (*n*=19)	Fellow PAC/PACS eyes (*n*=19)	*P*
Light Condition			
PD (mm)	2.901±0.931	2.446±0.446	0.014
IC (mm)	0.287±0.104	0.342±0.085	0.009
IT500 (mm)	0.397±0.082	0.385±0.079	0.466
IT750 (mm)	0.413±0.077	0.419±0.084	0.428
IT1000 (mm)	0.434±0.079	0.427±0.080	0.948
I-area (mm²)	1.718±0.256	1.871±0.236	0.001
Dark Condition			
PD (mm)	3.580±1.244	4.073±1.133	0.019
IC (mm)	0.298±0.083	0.325±0.075	0.048
IT500 (mm)	0.424±0.080	0.435±0.111	0.313
IT750 (mm)	0.441±0.082	0.481±0.084	0.006
IT1000 (mm)	0.480±0.092	0.511±0.098	0.034
I-area (mm²)	1.649±0.258	1.613±0.211	0.369

P=*P* of paired *t*-test between CPACG eyes and their fellow PAC/PACS eyes; CPACG=Chronic primary angle closure glaucoma; PAC=Primary angle closure; PACS=Primary angle closure suspect; PD=Pupil diameter; IC=Iris curvature; IT500=Iris thickness 500 µm from the iris root; IT750=Iris thickness 750 µm from the iris root; IT1000=Iris thickness 1000 µm from the iris root; I-area=Iris area

composition and anterior-segment parameters in patients with different stages of PAC. We found that type I collagen is less prevalent in CPACG eyes than in their fellow PAC/PACS eyes. In addition, decreased type I collagen density was correlated

Table 4: The light-to-dark changes of anterior segment measurements in recruited eyes

	CPACG eyes (*n*=19)	Fellow PAC/PACS eyes (*n*=19)	*P*
ΔACW (mm)	-0.065±0.345	-0.039±0.206	0.865
ΔACD (mm)	-0.011±0.034	-0.004±0.038	0.499
ΔACA (mm²)	-0.273±0.505	-0.867±0.522	**0.001**
ΔAOD (mm)	0.029±0.102	0.053±0.075	0.391
ΔARA (mm²)	0.006±0.028	0.015±0.025	0.279
ΔPD (mm)	-0.679±0.701	-1.627±0.802	**<0.001**
ΔIC (mm)	-0.011±0.065	0.021±0.066	0.075
ΔIT500 (mm)	-0.028±0.066	-0.063±0.085	0.099
ΔIT750 (mm)	-0.028±0.062	-0.068±0.067	0.055
ΔIT1000 (mm)	-0.045±0.064	-0.082±0.076	0.132
ΔI-area (mm²)	0.069±0.113	0.258±0.157	**0.001**
ΔLV (mm)	-0.007±0.127	0.021±0.105	0.947
ΔLT (mm)	0.018±0.357	-0.087±0.223	0.151

P=*P* of paired *t*-test between CPACG eyes and their fellow PAC/PACS eyes; CPACG=Chronic primary angle closure glaucoma; PAC=Primary angle closure; PACS=Primary angle closure suspect; Δ=The change of measurements from light to dark conditions (Light-Dark); ACW=Anterior chamber width; ACD=Anterior chamber depth; ACA=Anterior chamber cross-sectional area; AOD=Angle opening distance 500 µm from the scleral spur; ARA=Angle recess area 750 µm from the scleral spur; PD=Pupil diameter; IC=Iris curvature; IT500=Iris thickness 500 µm from the iris root; IT750=Iris thickness 750 µm from the iris root; IT1000=Iris thickness 1000 µm from the iris root; I-area=Iris area; LV=Lens vault; LT=Lens thickness

Table 5: Iris type I and type III collagen measurements in recruited eyes

	CPACG (*n*=19)	Fellow PAC/PACS eyes (*n*=19)	*P*
Collagen type I	0.069±0.025	0.087±0.031	**0.048**
Collagen type III	0.040±0.019	0.043±0.018	0.374
Type I/Type III	2.063±1.126	2.348±1.456	0.453

P=*P* of paired *t*-test between CPACG eyes and their fellow PAC/PACS eyes; CPACG=Chronic primary angle closure glaucoma; PAC=Primary angle closure; PACS=Primary angle closure suspect; Collagen type I=The ratio of cumulative areas of collagen type I fibers over the total cross-sectional areas. Collagen type III=The ratio of cumulative areas of collagen type III fibers over the total cross-sectional areas. Type I/type III=The ratio of cumulative areas of collagen type I fibers vs. collagen type III fibers

with decreased iris area in dark condition, decreased AOD and ARA under light condition and increased ACW under dark condition.

A previous study found that the density of type I collagen in iris tissues was lower in CPACG eyes than in primary open angle glaucoma (POAG), PACS, and age-matched normal eyes.[10] The current study showed that even in the same patient, decreased expression of type I collagen was associated with severity of disease. Type I collagen is the most prevalent collagen in iris tissues,[22] and it forms large fibers that contributes to its tensile strength.[23] We found that with the decrease of type I collagen, AOD and ARA under light condition and iris area in dark condition decreased in the current study. This and previous studies thus suggest that decrease of type I collagen content may result in a thin and floppy iris more prone to positional

Table 6: Correlations between Iris Type I Collagen Measurements and Anterior Segment Parameters under Light and Dark Conditions in Recruited Eyes

	Light Condition		Dark Condition	
	r	*P*	*r*	*P*
ACW (mm)	-0.406	**0.029**	-0.484	0.007
ACD (mm)	0.027	0.887	0.027	0.885
ACA (mm²)	-0.121	0.518	-0.177	0.342
AOD (mm)	0.402	**0.031**	0.134	0.481
ARA (mm²)	0.442	**0.018**	0.294	0.108
PD (mm)	-0.181	0.330	-0.288	0.217
IC (mm)	0.037	0.847	0.298	0.110
IT500 (mm)	-0.029	0.882	-0.320	0.085
IT750 (mm)	0.069	0.721	-0.064	0.736
IT1000 (mm)	0.130	0.501	0.025	0.894
IA (mm2)	0.298	0.107	0.387	**0.032**
LV (mm)	-0.260	0.172	-0.206	0.275
LT (mm)	-0.048	0.809	-0.149	0.440

r=Pearson correlation coefficients; ACW=Anterior chamber width; ACD=Anterior chamber depth; ACA=Anterior chamber cross-sectional area; AOD=Angle opening distance 500 μm from the scleral spur; ARA=Angle recess area 750 μm from the scleral spur; PD=Pupil diameter; IC=Iris curvature; IT500=Iris thickness 500 μm from the iris root; IT750=Iris thickness 750 μm from the iris root; IT1000=Iris thickness 1000 μm from the iris root; IA=Iris cross-sectional area; LV=Lens vault; LT=Lens thickness

changes effected by aqueous currents, and thus may aggravate pupillary block and synechial closure.

One previous study found that the density of type I collagen was higher in irises of eyes with acute angle closure (AAC) and their fellow eyes than those in POAG, PACS, and age-matched normal eyes.[10] In addition, in patients with PACG (some of whom had experienced acute attack, and most had laser peripheral iridotomy performed), iris type I collagen expressions was higher than those in POAG patients and controls.[11] Another study found that gene expressions of Col1A1, which codes for the pro-α1(I) chain component of type I collagen, in patients with PACG were higher than those in patients with POAG.[24] These discrepant results imply that the mechanism of acute attack of PAC may be different from chronic development of PACG. Moreover, laser iridotomy may also lead to histological changes of the iris in angle closure.[25,26] In addition, the PACG eye and the PAC/PACS eye in the same patient were compared in the current study, which reflect the effect of severity of disease on the iris. The above studies compared PACG eyes with POAG eyes and/or control eyes, which reflect the effect of type of glaucoma on the iris. Alternatively, the decrease in iris thickness and density of type I collagen, less light-to-dark changes in iris area may be a consequence of chronically elevated intraocular pressure and the resultant ischemic iris atrophy in CPACG patients. Several factors such as chronic and intermittent adhesion between peripheral iris and the trabecular meshwork/peripheral cornea, and intermittent decreased blood flow owing to transient, less pronounced IOP spikes may affect the expression of collagen in the iris.

Our study found that with the decrease of type I collagen density, ACW under dark condition increased. Type I collagen exists not only in the iris, but also the sclera, where it plays important role in determining scleral strength[27] and plasticity.[22] The increase of ACW in PACG eyes may be an indirect evidence of altered expression of collagen in the sclera, affecting the severity and course of disease.

There are several limitations to the current study: (1) It is a cross-sectional study comparing PACG eyes with their fellow eyes with PAC/PACS; no control group was set. However, it was difficult to find age-matched control group to perform both *in vivo* and *in vitro* measurements. (2) The semi-quantitative assessment of collagen fibers was somewhat arbitrary. However, sirius red is a selective staining method for the differentiation of type I and type III collagen,[28] which has been widely used in quantitative studies on liver cirrhosis, lung, and iris tissues.[10,29,30] (3) Because of the strict inclusion criteria of the current study, the sample size was relatively small. Nevertheless, statistically significant differences had been found.

Conclusion

In summary, decreased type I collagen density in iris tissues is correlated with decreased iris area, less light-to-dark changes in pupil diameter and iris area in CPACG eyes compared with their fellow PAC/PACS eyes. Although it is difficult to differentiate between correlation and causation in this cross-sectional comparison study, the data do offer some clues on the structural reasons for the biometric and dynamic differences in the iris that may contribute to the disease progression of PAC.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No 81670850) and the Research Grant from Guangzhou Science Technology and Innovation Commission in China (Grant No 201607010321).

Financial support and sponsorship

This research was supported by the National Natural Science Foundation of China (Grant No 81670850) and the Research Grant from Guangzhou Science Technology and Innovation Commission in China (Grant No 201607010321).

Conflicts of interest

There are no conflicts of interest.

References

1. Cook C, Foster P. Epidemiology of glaucoma: What's new? Can J Ophthalmol 2012;47:223-6.
2. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006;90:262-7.
3. Wang YE, Li Y, Wang D, He M, Lin S. Comparison of factors associated with occludable angle between American Caucasian and ethnic Chinese. Invest Ophthalmol Vis Sci 2013;54:7717-23.
4. Aung T, Nolan WP, Machin D, Seah SK, Baasanhu J, Khaw PT, *et al.* Anterior chamber depth and the risk of primary angle closure in 2 East Asian populations. Arch Ophthalmol 2005;123:527-32.
5. Nongpiur ME, He M, Amerasinghe N, Friedman DS, Tay WT, Baskaran M, *et al.* Lens vault, thickness, and position in Chinese subjects with angle closure. Ophthalmology 2011;118:474-9.
6. Narayanaswamy A, Zheng C, Perera SA, Htoon HM, Friedman DS, Tun TA, *et al.* Variations in iris volume with physiologic mydriasis in subtypes of primary angle closure glaucoma. Invest Ophthalmol Vis Sci 2013;54:708-13.
7. Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and

classification of glaucoma in prevalence surveys. Br J Ophthalmol 2002,86:238-42.

8. Huang J, Wang Z, Wu Z, Li Z, Lai K, Ge J. Comparison of ocular biometry between eyes with chronic primary angle-closure glaucoma and their fellow eyes with primary angle-closure or primary angle-closure suspect. J Glaucoma 2015;24:323-7.
9. Lin J, Wang Z, Chung C, Xu J, Dai M, Huang J. Dynamic changes of anterior segment in patients with different stages of primary angle-closure in both eyes and normal subjects. PLoS One 2017;12:e0177769.
10. He M, Lu Y, Liu X, Ye T, Foster PJ. Histologic changes of the iris in the development of angle closure in Chinese eyes. J Glaucoma 2008;17:386-92.
11. Chua J, Seet LF, Jiang Y, Su R, Htoon HM, Charlton A, *et al.* Increased SPARC expression in primary angle closure glaucoma iris. Mol Vis 2008;14:1886-92.
12. Chinese Glaucoma Society. Consensus of the diagnosis and treatment of primary glaucoma in China (2014). Zhonghua Yan Ke Za Zhi 2014;50:382-3.
13. Chew P, Sng C, Aquino MC, See J. Surgical treatment of angle-closure glaucoma. Dev Ophthalmol 2012;50:137-45.
14. Savelsbergh-Fillette MP, Demailly P. Surgical peripheral iridectomy and argon laser iridotomy in primary closed-angle glaucoma. Comparative statistical study. J Fr Ophtalmol 1988;11:349-56.
15. Gao XB, Zhang XL, Chen G, Huang XK, Zhong XJ, Lin MK, *et al.* The blood-aqueous barrier changes after laser peripheral iridotomy or surgery peripheral iridectomy. Zhonghua Yan Ke Za Zhi 2011;47:876-80.
16. Li X, Wang W, Huang W, Chen S, Wang J, Wang Z, *et al.* Difference of uveal parameters between the acute primary angle closure eyes and the fellow eyes. Eye 2018;32:1174-82.
17. Seager FE, Jefferys JL, Quigley HA. Comparison of dynamic changes in anterior ocular structures examined with anterior segment optical coherence tomography in a cohort of various origins. Invest Ophthalmol Vis Sci 2014;55:1672-83.
18. Wang Z, Liang X, Wu Z, Lin J, Huang J. A novel method for measuring anterior segment area of the eye on ultrasound biomicroscopic images using photoshop. PLoS One 2015;10:e0120843.
19. Trope GE. Glaucoma Surgery. FL, USA: Taylor & Francis Group; 2005.
20. Quigley HA. Angle-closure glaucoma-simpler answers to complex mechanisms: LXVI Edward Jackson Memorial Lecture. Am J Ophthalmol 2009;148:657-69.e1.
21. Quigley HA. The iris is a sponge: A cause of angle closure. Ophthalmology 2010;117:1-2.
22. Konstas AG, Marshall GE, Lee WR. Immunocytochemical localisation of collagens (I-V) in the human iris. Graefes Arch Clin Exp Ophthalmol 1990;228:180-6.
23. Goldbaum MH, Jeng S, Logemann R, Weinreb RN. The extracellular matrix of the human optic nerve. Arch Ophthalmol 1989;107:1225-31.
24. Seet LF, Narayanaswamy A, Finger SN, Htoon HM, Nongpiur ME, Toh LZ, *et al.* Distinct iris gene expression profiles of primary angle closure glaucoma and primary open angle glaucoma and their interaction with ocular biometric parameters. Clin Exp Ophthalmol 2016;44:684-92.
25. Goldberg MF, Tso MO, Mirolovich M. Histopathological characteristics of neodymium-YAG laser iridotomy in the human eye. Br J Ophthalmol 1987;71:623-8.
26. Tetsumoto K, Küchle M, Naumann GO. Late histopathological findings of neodymium: YAG laser iridotomies in humans. Arch Ophthalmol 1992;110:1119-23.
27. Watson PG, Young RD. Scleral structure, organisation and disease. A review. Exp Eye Res 2004;78:609-23.
28. Junqueira LC, Cossermelli W, Brentani R. Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. Arch Histol Jpn 1978;41:267-74.
29. James J, Bosch KS, Aronson DC, Houtkooper JM. Sirius red histophotometry and spectrophotometry of sections in the assessment of the collagen content of liver tissue and its application in growing rat liver. Liver 1990;10:1-5.
30. Armendáriz-Borunda J, Rojkind M. A simple quantitative method for collagen typing in tissue samples: Its application to human liver with schistosomiasis. Coll Relat Res 1984;4:35-47.