Deep Retinal Capillary Plexus Decreasing Correlated With the Outer Retinal Layer Alteration and Visual Acuity Impairment in Pathological Myopia

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Purpose. The purpose of this study was to measure alterations of inner retinal microvascular density and outer retinal sublayer thicknesses in pathological myopia, and to correlate the measured parameters with best corrected visual acuity (BCVA).

Methods. Optical coherence tomography (OCT) and OCT angiography (OCTA) images of 21 control, 48 simple high myopia, and 22 pathological myopia eyes were analyzed to quantify the thicknesses of the outer retinal sublayers and the density of the inner retinal microvascular network that includes the superficial retinal capillary plexus (SRCP) and deep retinal capillary plexus (DRCP). Retinal sublayer thicknesses and microvascular densities were compared among the three groups, and correlations of sublayer thicknesses and microvascular densities with BCVA were determined.

Results. In pathological myopia, density of the DRCP, thicknesses of myoid and ellipsoid zone (MEZ), interdigitation zone and retinal pigment epithelium/Bruch complex (IZ + RPE), and choroid were lower than in simple high myopia ($P < 0.05$). The decreased DRCP density was correlated with thinner MEZ and IZ+RPE in pathological myopia ($P < 0.05$), but not in simple high myopia ($P > 0.05$). Simple linear regression showed that axial length, female, thicknesses of outer plexiform layer (OPL), MEZ, IZ + RPE, choroid, and density of the SRCP and DRCP were correlated with BCVA. In multiple regression analysis, worse BCVA was associated only with thinner MEZ, thinner choroid, and decreased DRCP ($P < 0.05$).

Conclusions. Alteration of inner retinal microvascular density and outer retinal sublayer thicknesses occurred in pathological myopia, especially decreased DRCP and thinner MEZ, which were significantly associated with worse BCVA.

Keywords: pathological myopia, optical coherence tomography, visual impairment, retinal microvasculature, photoreceptor dystrophy

With the increasing prevalence of myopia, high myopia is one of the leading causes of visual impairment that affects more than 163 million people worldwide.[1] Pathological myopia is characterized by degeneration of the chorioretinal structure and vasculature that results in vision impairment, even blindness. Thus, studies of the early changes in the chorioretinal structure and vasculature, as well as the interactions between them, may have great significance in exposing the pathogenic mechanism of visual impairment and in advancing new strategies for the treatment of pathological myopia.

With advances of imaging techniques and related image analysis algorithms, our recent studies demonstrated that disruption of the photoreceptors was one of the most important factors associated with visual impairment in high myopia.[2,3] As cone photoreceptors are located mainly in the avascular outer retina, the oxygen requirements of this region depend primarily on diffusion from the choroid.[4,5] Moreover, recent studies demonstrated that the inner retinal microvasculature supplies oxygen to the outer retinal layer as well.[6,7] Therefore, in cases where choroidal perfusion becomes significantly reduced in high myopia, the inability of the inner retinal microvasculature to supply sufficient oxygen to the outer retina potentially contributes to the worsening of photoreceptor degeneration and consequent impairment of visual acuity, which has never been documented.

Previously, we developed custom algorithms that automatically identify the thickness of outer retinal structures and the density of the retinal microvasculature in optical coherence tomography (OCT) and OCT angiography (OCTA) images.[8,9] In the current study, we evaluated changes in the outer retinal sublayer thicknesses imaged by OCT and alterations of the inner retinal microvascular density imaged by OCTA in simple high myopia and pathological myopia. We also assessed the associations of these changes with visual impairment.

Methods

Study Design

This was a retrospective, cross-sectional study and was approved by the Ethics Committee of Wenzhou Medical University, Wenzhou, Zhejiang, China, in accordance with the tenets of the Declaration of Helsinki.

Subject Selection

The records of all subjects who were recruited from February 2018 to September 2018 at the Affiliated Eye Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China, were examined. The enrolled subjects were divided into the following three groups: (1) control group with a spherical equivalent (SE) ranged from −1.0 diopters (Ds) to +1.0 (Ds), (2) simple high myopia with an SE less than or equal to −6.0 Ds, or axial length (AL) ≥26.5 mm, and without myopic maculopathy, and (3) pathological myopia with an SE less than or equal to −6.0 D, or AL ≥26.5 mm, and with myopic maculopathy. According to the International Meta-Analysis for Pathologic Myopia (META-PM) classification system, eyes with diffuse chorioretinal atrophy, patchy chorioretinal atrophy, or macular atrophy were considered as having pathological myopia.[10] Two ophthalmologists diagnosed and categorized each simple high myopia and pathological myopia case. If the diagnosis from these two ophthalmologists were not in accord with each other, the final judgement was made by another senior ophthalmologist. Eyes with intraocular pressure (IOP) >21 mm Hg, significant cataract, diabetic retinopathy, age-related macular degeneration, glaucoma, a history of intraocular surgery, serious complications of high myopia, such as retinoschisis and choroidal neovascularization, or related systemic diseases were excluded from the current study.

Clinical Examinations

All subjects received a comprehensive clinical ophthalmologic examination that included refraction, best corrected visual acuity (BCVA) measured as the logarithm of the minimum angle of resolution (LogMAR), and slit lamp biomicroscopy. The SE of the refractive error was defined as the spherical dioptric power plus one-half of the cylindrical dioptric power. AL was measured with an IOL Master 500 (Carl Zeiss, Jena, Germany). Non-contact IOP was measured by a Full Auto Tonometer TX-F (Topcon, Tokyo, Japan), and fundus photographs (Fig. 1A) were taken with a 45-degree digital retinal camera (Canon EOS 10D SLR backing; Canon, Inc., Tokyo, Japan).

Image Acquisition Protocol and Analysis

All enrolled subjects were imaged by OCT (Optovue RTVue XR Avanti; Optovue, Inc., Fremont, CA, USA) to obtain structural OCT and OCTA images. Transverse section images of the fundus were obtained by radial line (with the length of 8 mm for the eyes with the AL of 24.46 mm) scans passing through the fovea, captured in 18 consecutive B-scans. These images were then used to build the macular three-dimensional topographic map. The OCTA images were obtained by orthogonal registration and merging of two consecutive scans (with the size of 3 × 3 mm for the eyes with the AL of 24.46 mm) centered on the fovea. Good sets of scans with a signal strength index >4/10 for OCTA image and >40/100 for OCT B-scan image (defined by the machine) were selected for further analysis. Images with motion artifacts were excluded.

The OCTA images were corrected for image magnification based on the AL using Bennett's formula, $t = p \times q \times s$ (t, the real scan range; p, the magnification factor determined by the imaging system camera; q, the magnification factor related to the eye; s, the original measured range from the imaging system), which was reported in our previous papers.[2,11] Briefly, the q was determined by the equation $q = 0.01306 \times (\text{AL} - 1.82)$, the scan range t then was equaled to $(\text{AL} - 1.82) / 22.64 \times s$. The custom-developed software was then used to quantify the vessel density for the superficial retinal capillary plexus (SRCP) and the deep retinal capillary plexus (DRCP). The SRCP extends from the internal limiting membrane to 10 µm above the inner plexiform layer (Fig. 1B). The DRCP extends from 10 µm above the inner plexiform layer to the 10 µm below the outer plexiform layer (OPL) (Fig. 1C). The density of the SRCP and DRCP were automatically calculated for the macular region with a diameter of 2.5 mm, excluding the foveal avascular zone (FAZ). The FAZ lies within a circle of fixed radius (diameter = 0.8 mm) at the center of the fovea. Details about the software were reported in a previous paper.[9]

Bennett's formula was also used to correct the magnification of OCT B-scan images based on the AL. Following magnification correction, another custom-developed software was used to quantify the outer retinal sublayer thicknesses and the choroidal thickness in the macular region with a diameter of 2.5 mm that included (1) the OPL, (2) the Henle fiber layer and outer nuclear layer (HFL + ONL), (3) the myoid and ellipsoid zone (MEZ), (4) the outer segment of photoreceptors (OS), (5) the interdigitation zone and retinal pigment epithelium/Bruch complex (IZ + RPE), and (6) the choroid (Fig. 1D). Based on the gradient information and shortest path search, the principle and use of the algorithm identified the different layers in the whole target region automatically and analyzed layer thicknesses, which were demonstrated in our previous papers.[8,12] The data reported from this software was the average thickness in our target analyzed region. When the automated segmentation was not correct (e.g. with small peaks and curve offsets), we made manual corrections. All segmentation analyses were done by one masked reader.

Two separate sets of OCT B-scan images and another two separate sets of OCTA images were collected and then the repeatability tests were done for the outer retinal thicknesses, choroidal thickness, and inner retinal microvasculature density in three groups (10 eyes in each group). The intraclass correlation (ICC) and Bland-Altman plots were analyzed.

Statistical Analyses

All data were calculated as means ± SDs and analyzed with SPSS software (version 22.0; SPSS, Inc., Chicago, IL, USA). Differences of sex among the three groups were determined by the χ^2 test. One-way analysis of variance (ANOVA) and post hoc had done to compare the differences among the three groups. General estimating equation was used to adjust the sex influence on the comparisons of the outer retinal and choroidal thickness, and inner retinal microvascular density among the three groups. Pearson's correlation, partial correlation, and linear regression were used to calculate relation-

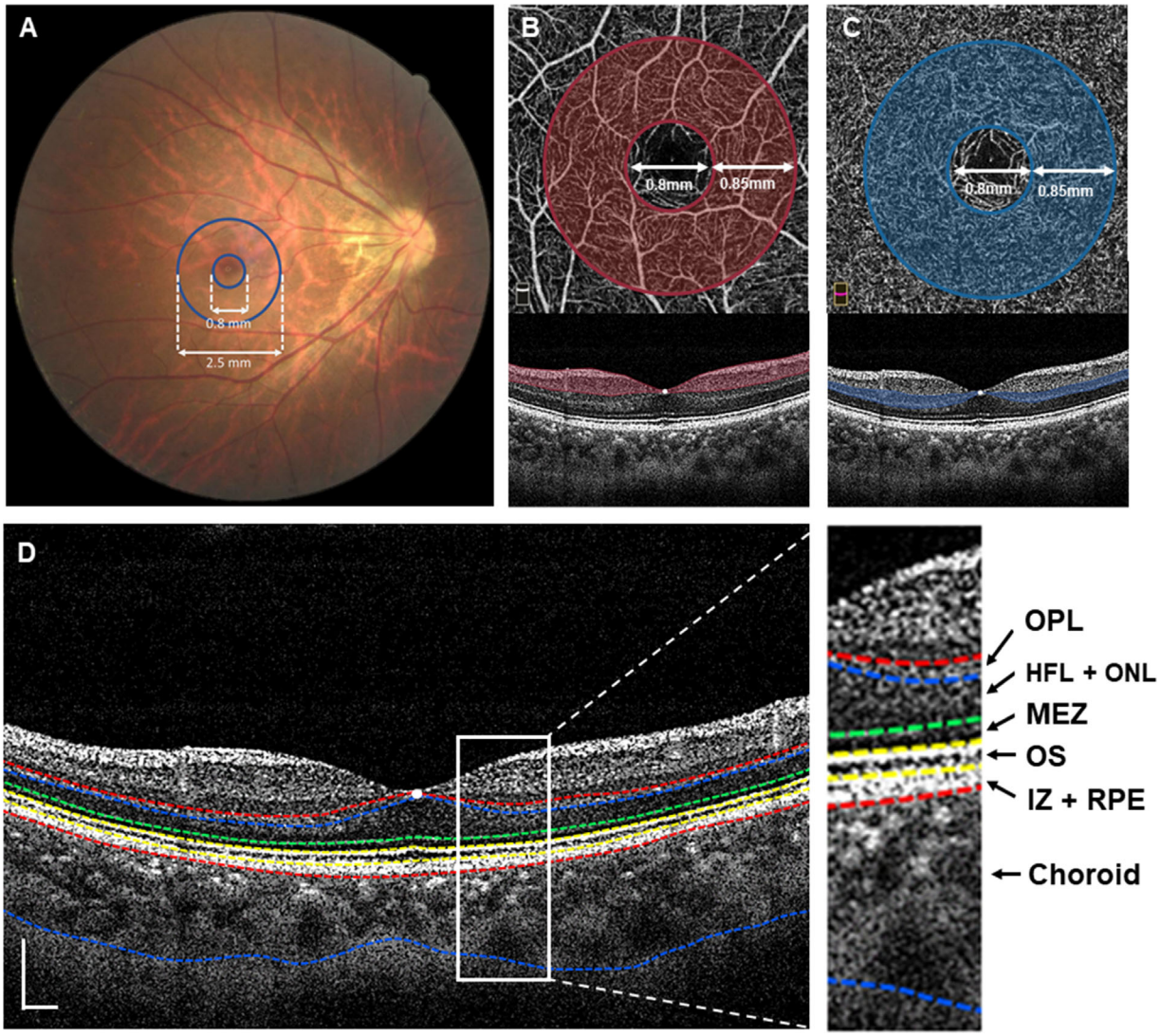

Figure 1. Fundus photographs and optical coherence tomography (OCT)/OCT angiography (OCTA) images analysis. (**A**) Fundus photographic image with the macula divided in two subfields: the FAZ (diameter = 0.8 mm) and the outer ring (diameter = 2.5 mm). (**B**) OCTA image of the SRCP with its corresponding area in the B-scan image. (**C**) OCTA image of DRCP with its corresponding area in the B-scan image. (**D**) OCT image of the macula. Bar = 300 µm.

ships among the AL, outer retinal sublayer and choroidal thicknesses, the densities of the macular SRCP and DRCP, and BCVA. The *P* values < 0.05 were considered statistically significant.

Results

Basic Patient Characteristics

We excluded the patients with pathological myopia in category four of the META-PM to avoid the condition of the no vessel in the atrophic area. A total of 52 patients with pathological myopia were done using the OCT/OCTA examination. Then a masked reader reviewed all images to exclude images with low quality, obvious motion artifact and, especially, images showing patchy atrophy or worse in the target analyzed area. Finally, a total of 22 patients with pathological myopia were included for further analysis in current research. There were no significant differences in sex, age, or IOP among the three groups ($P = 0.259$, 0.076, and 0.192, respectively; Table 1). However, compared to patients with simple high myopia, patients with pathological myopia had greater refractive errors, worse visual acuities, and longer axial lengths ($P < 0.001$ for all; Table 1).

Differences in Outer Retinal Thicknesses and Inner Retinal Microvasculature Density Among the Three Groups

The representative and variative fundus photographs and OCT/OCTA images among the three groups are shown in Figure 2, Supplementary Figures S1, S2 and S3. During the two repeated measurements, the ICC of outer retinal sublayers and choroidal thicknesses varied from 0.896 to 0.996 in three groups, and ICC of inner retinal microvascular density varied from 0.861 to 0.956 (Table 2). The Bland-Altman plots showed that most outer retinal thickness and

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Table 1. Basic Characteristics of the Three Groups

Parameters	Control Group	Simple High Myopia	Pathological Myopia	*P* Value*	*P* Value$_1$	*P* Value$_2$	*P* Value$_3$
N	21	48	22	–	–	–	–
Age, year	37.5 ± 13.6	32.4 ± 9.1	37.7 ± 11.4	0.076	0.331	1.000	0.175
Sex, F:M	15:6	30:18	18:4	0.259	0.474	0.420	0.106
IOP, mm Hg	14.44 ± 3.98	15.97 ± 2.44	14.76 ± 4.02	0.192	0.111	0.769	0.181
SE, Diopter	−0.51 ± 0.87	−10.31 ± 2.67	−13.88 ± 3.25	<0.001	<0.001	<0.001	<0.001
BCVA, LogMAR	−0.02 ± 0.04	0.02 ± 0.03	0.16 ± 0.16	<0.001	0.104	<0.001	<0.001
AL, mm	24.05 ±0.74	27.65 ± 1.16	29.21 ± 1.24	<0.001	<0.001	<0.001	<0.001

–, not performed; F, female; M, male.
* *P* value among the three groups; *P* Value$_1$, *P* value between control group and simple high myopia; *P* Value$_2$, *P* value between control group and pathological myopia; *P* Value$_3$, *P* value between simple high myopia and pathological myopia.

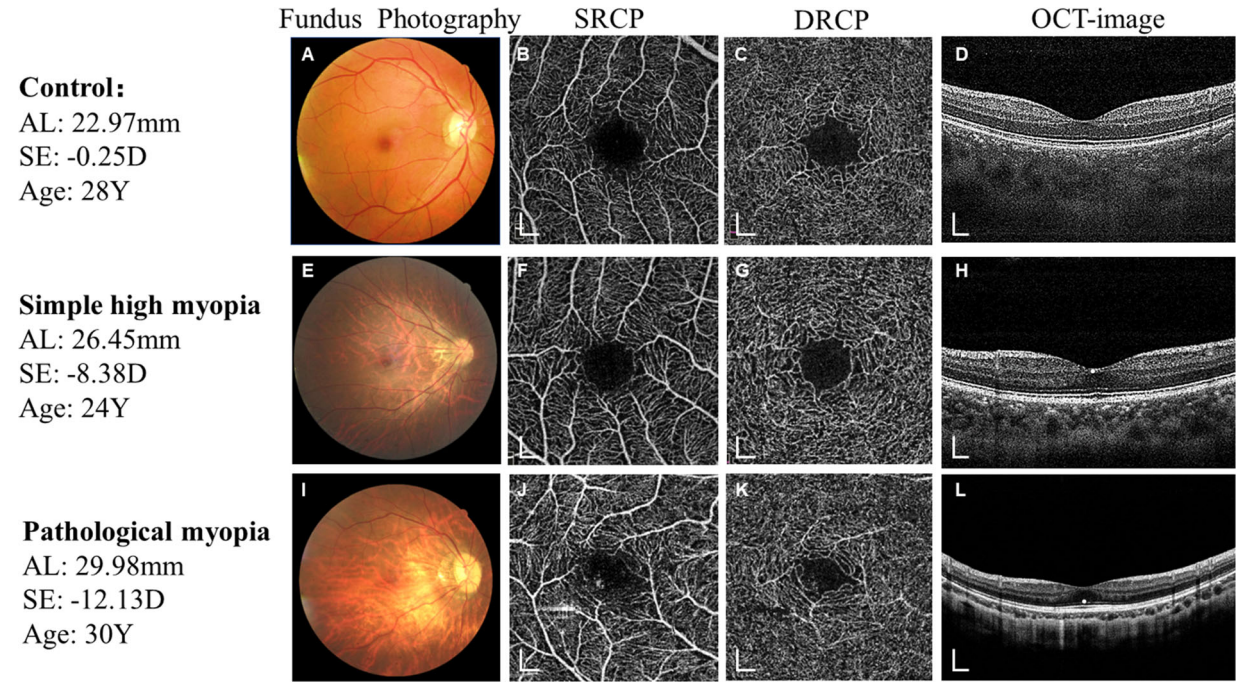

Figure 2. Representative fundus photographs and optical coherence tomography (OCT)/OCT angiography (OCTA) images of control, simple high myopia, and pathological myopia eyes. (**A**) Fundus photographic image in control group. (**B**) OCTA image of the SRCP in control group. (**C**) OCTA image of the DRCP in control group. (**D**) OCT image of the macula in control group. (**E**) Fundus photographic image of simple high myopia. (**F**) OCTA image of the SRCP in simple high myopia. (**G**) OCTA image of the DRCP in simple high myopia. (**H**) OCT image of the macula in simple high myopia. (**I**) Fundus photographic image of pathological myopia. (**J**) OCTA image of the SRCP in pathological myopia. (**K**) OCTA image of the DRCP in pathological myopia. (**L**) OCT image of the macula in pathological myopia. Bars = 300 µm.

Table 2. The Intraclass Correlation of Outer Retinal and Choroidal Thicknesses, and Inner Retinal Microvascular Density of Eyes Among the Three Groups

Parameters	Control Group	Simple High Myopia	Pathological Myopia
Outer retinal sublayers and choroidal thickness			
OPL	0.951	0.896	0.962
HFL + ONL	0.976	0.975	0.968
MEZ	0.974	0.988	0.971
OS	0.897	0.965	0.996
IZ + RPE	0.931	0.951	0.985
Choroid	0.942	0.990	0.984
Inner retinal microvascular density			
SRCP	0.909	0.862	0.861
DRCP	0.956	0.946	0.938

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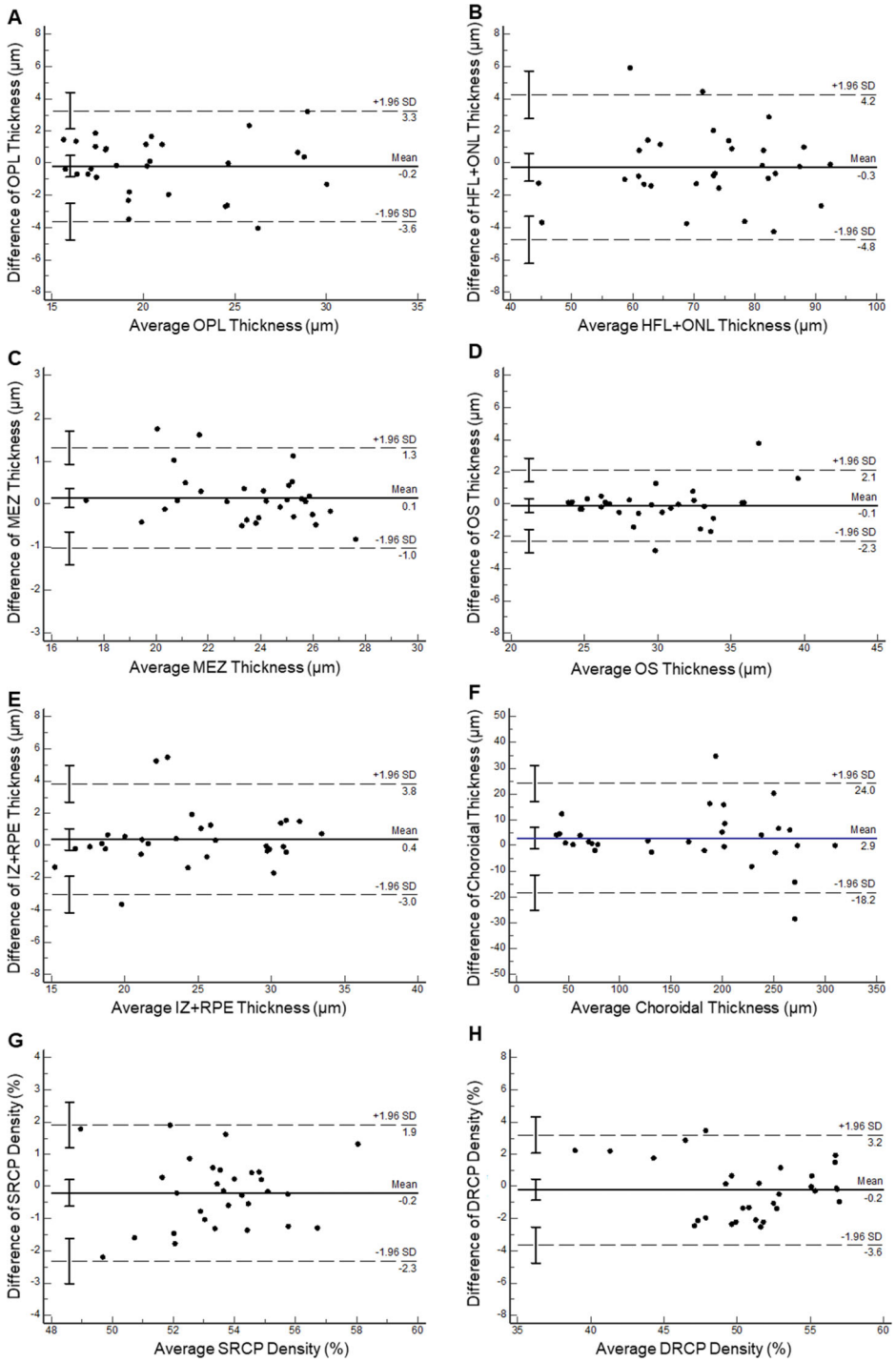

Figure 3. Bland-Altman analysis for two repeated measurements of the outer retinal and choroidal thicknesses, and inner retinal microvascular density in all groups (including control group, simple high myopia, and pathological myopia). (**A**) OPL; (**B**) HFL + ONL; (**C**) MEZ; (**D**) OS; (**E**) IZ + RPE; (**F**) choroid; (**G**) SRCP; and (**H**) DRCP. Solid lines and dashed lines represent mean differences of two repeated measurements and limits of agreement. Error bars show 95% confidence interval of mean difference and limits of agreement.

inner retinal microvascular density's differences between the two repeated measurements were within the limits of agreement (Fig. 3).

Among the three groups, the outer retinal and choroidal thicknesses were significantly different ($P < 0.04$; Table 3). The outer retinal sublayers thicknesses in eyes with simple high myopia were all different from those with pathological myopia except for the OS and HFL + ONL layers (Table 3, Fig. 4A). The OPL in the simple high myopia eyes, 19.3 ± 3.4 μm, was thinner compared to the pathological myopia eyes, 21.2 ± 3.8 μm ($P = 0.047$; Table 3). The MEZ and IZ + RPE in simple high myopia eyes were thicker than in pathological myopia eyes ($P = 0.002$ and 0.001, respectively). Further, the choroid was significantly thicker in eyes

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Table 3. Outer Retinal and Choroidal Thicknesses and Inner Retinal Microvascular Density of Eyes Among the Three Groups

Parameters	Control Group	Simple High Myopia	Pathological Myopia	*P* Value*	P Value$_1$	P Value$_2$	*P* Value$_3$
Outer retinal sublayers and choroidal thickness							
OPL, μm	18.4 ± 3.9	19.3 ± 3.4	21.2 ± 3.8	0.039 (0.047)	0.351 (0.262)	0.014 (0.015)	0.047 (0.083)
HFL + ONL, μm	82.0 ± 5.6	77.4 ± 9.0	70.3 ± 11.8	<0.001 (<0.001)	0.042 (0.030)	0.001 (<0.001)	0.051 (0.007)
MEZ, μm	23.4 ± 1.8	23.1 ± 2.1	21.5 ± 1.7	0.002 (0.002)	0.541 (0.251)	0.002 (0.001)	0.002 (0.005)
OS, μm	26.9 ± 2.7	31.1 ± 4.5	30.8 ± 4.5	0.001 (<0.001)	<0.001 (<0.001)	0.004 (0.002)	0.994 (0.705)
IZ + RPE, μm	32.0 ± 3.1	26.4 ± 4.3	22.5 ± 3.4	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)	0.001 (<0.001)
Choroid, μm	256.2 ± 53.7	159.8 ± 49.0	75.9 ± 19.7	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)	0.001 (<0.001)
Inner retinal microvascular density							
SRCP, %	55.8 ± 1.6	53.8 ± 2.0	52.8 ± 1.9	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)	0.116 (0.053)
DRCP, %	54.5 ± 2.5	51.8 ± 4.2	47.0 ± 3.9	<0.001 (<0.001)	0.004 (0.035)	<0.001 (<0.001)	0.001 (<0.001)

* *P* value among the three groups; *P* value$_1$, *P* value between control group and simple high myopia; *P* value$_2$, *P* value between control group and pathological myopia; *P* value$_3$, *P* value between simple high myopia and pathological myopia; Values in parentheses were the *P* values after the adjustment for the sex.

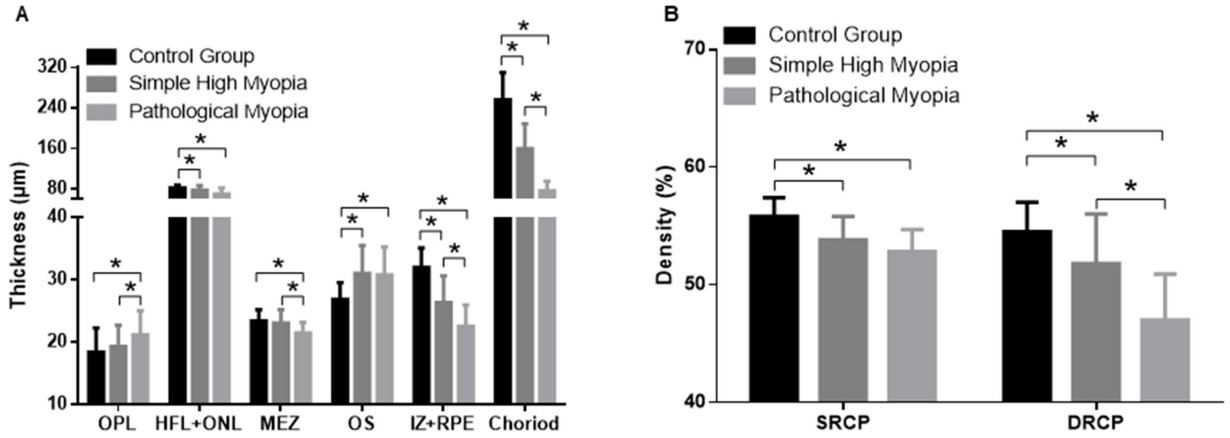

Figure 4. Thickness of outer retinal sublayers and choroid, and the inner retinal microvasculature among the three groups. (**A**) Thickness of the outer retinal sublayers and the choroid. (**B**) Inner retinal microvasculature. *$P < 0.05$, without adjustment for sex.

with simple high myopia compared to eyes with pathological myopia ($P < 0.001$; Fig. 4A). When adjusted for sex, the alteration tendency of the outer retinal and choroidal thickness was similar as seen before the adjustment (Table 3).

The significant differences of the inner retinal microvascular density among the three groups were found ($P < 0.001$; Table 3). For the inner retinal microvascular density, there was no significant difference in the SRCP density between the simple high myopia and pathological myopia ($P = 0.116$; Table 3, Fig. 4B). However, the DRCP density in eyes with simple high myopia, 51.8 ± 4.2%, was higher than in pathological myopia, 47.0 ± 3.9% ($P = 0.001$; Table 3, Fig. 4B). Moreover, the similar alteration tendency of the inner retinal microvascular density was found no matter with and without adjustment for the sex (Table 3).

Correlation Between Outer Retinal Sublayer Thicknesses and the Deep Retinal Capillary Plexus Density

For simple high myopia, none of the outer retinal sublayer thicknesses were correlated with DRCP density (Table 4). However, the thickness of MEZ and OS were significantly correlated with the DRCP density in eyes with pathological myopia, with or without adjustment for AL (all P < 0.05, Table 4, Fig. 5). In pathological myopia, the thickness of the IZ + RPE was correlated with the DRCP density only after adjusting the axial length ($P = 0.040$). Among the significantly correlated layers, only the OS was negatively correlated with DRCP density.

Associations Among Intraretinal Thickness, Inner Retinal Microvascular Density, and BCVA

Based on the collective data for all patients with high myopia, simple linear regression showed that longer AL ($P < 0.001$), women ($P = 0.021$), thicker OPL ($P = 0.016$), thinner MEZ ($P < 0.001$), thinner IZ + RPE ($P < 0.001$), thinner

Table 4. Pearson's Correlation between the Outer Retinal Sublayer Thicknesses (μm) and the Deep Retinal Capillary Plexus Density (%)

	Simple High Myopia			Pathological Myopia		
Layer	*r*	*P* Value	*P* Value*	*r*	*P* Value	*P* Value*
OPL	−0.123	0.206	0.285	−0.341	0.107	0.095
HFL + ONL	−0.062	0.338	0.395	0.476	0.037	0.052
MEZ	0.105	0.241	0.099	0.597	**0.009**	**0.003**
OS	−0.177	0.117	0.182	−0.574	**0.013**	**0.005**
IZ + RPE	0.060	0.345	0.372	0.392	0.074	**0.040**
Choroid	0.146	0.164	0.426	0.156	0.290	0.246

* Partial correlation with adjustment for axial length.

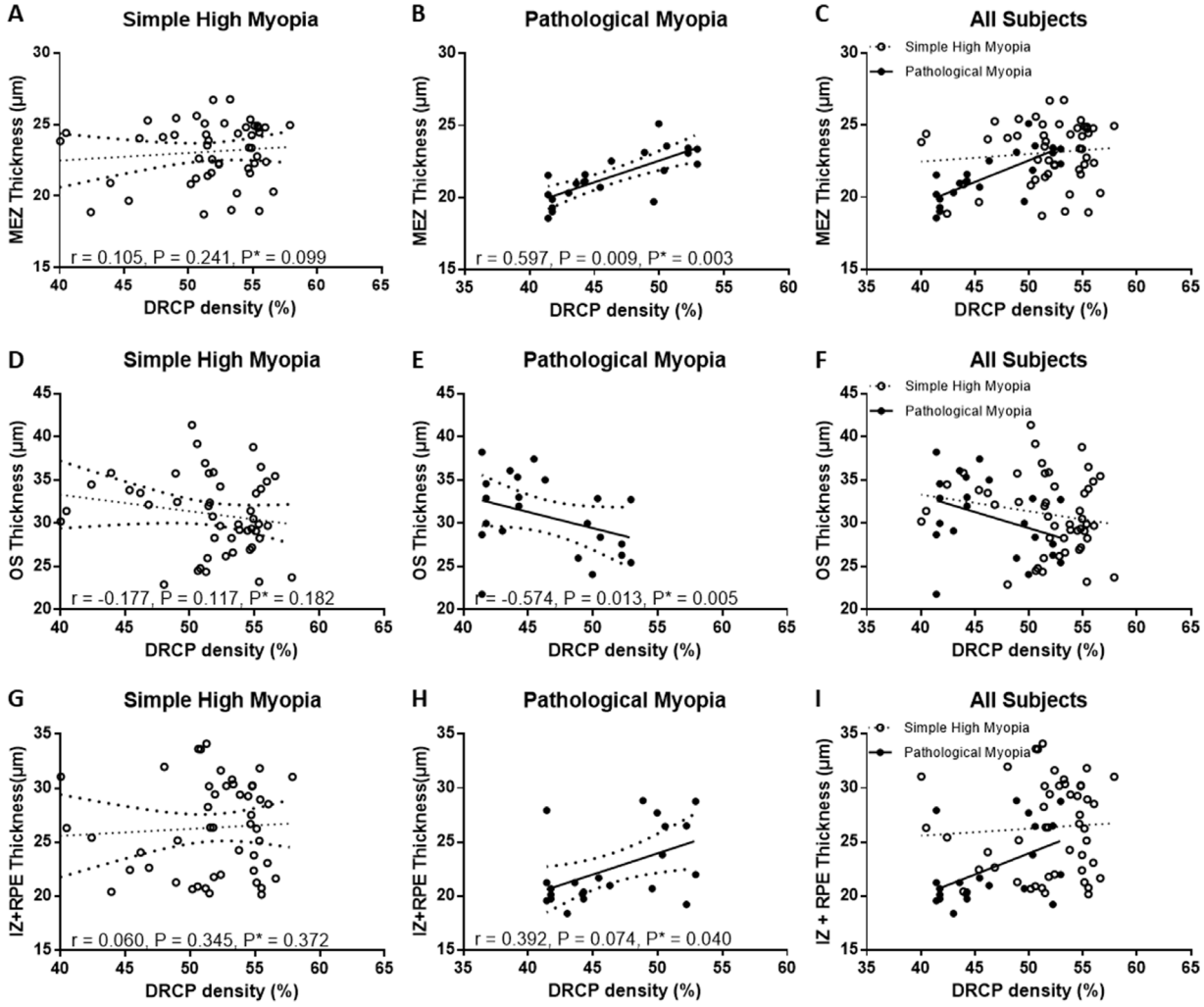

Figure 5. Correlation between DRCP density and the outer retinal sublayer thickness in simple high myopia and pathological myopia. (**A**) Correlation between DRCP density and MEZ thickness in simple high myopia. (**B**) Correlation between DRCP density and MEZ thickness in pathological myopia. (**C**) Scatter diagram between DRCP density and MEZ thickness in all high myopia subjects. (**D**) Correlation between DRCP density and OS thickness in simple high myopia. (**E**) Correlation between DRCP density and OS thickness in pathological myopia. (**F**) Scatter diagram between DRCP density and OS thickness in all high myopia subjects. (**G**) Correlation between DRCP density and IZ + RPE thickness in simple high myopia. (**H**) Correlation between DRCP density and IZ + RPE thickness in pathological myopia. (**I**) Scatter diagram between DRCP density and IZ + RPE thickness in all high myopia subjects. P*, *P* value after adjusting the axial length by partial correlation. The dashed lines are the 95% confidence intervals for the regression lines.

choroid ($P < 0.001$), lower density of SRCP ($P = 0.001$), and lower density of DRCP ($P < 0.001$) were significantly associated with worse BCVA (Table 5). The thicknesses of the HFL+ ONL and the OS were not correlated with the BCVA ($P = 0.063$ and 0.055, respectively).

The parameters that were significantly correlated with the BCVA (Table 5) were included in the multiple regression analysis (Table 5). For the final multiple regression with BCVA as the outcome, only the thickness of MEZ ($P = 0.010$), choroid ($P = 0.008$), and DRCP density ($P = 0.003$) were significant factors for the BCVA.

Discussion

In this study, we used OCT and OCTA to compare changes in the outer retinal sublayer thicknesses and changes in the inner retinal microvasculature of eyes with simple high myopia and with pathological myopia. We also determined the (1) correlations of the outer retinal layer thicknesses with the inner retinal microvascular density and (2) the correlations of the outer retinal layer thicknesses and the inner retinal microvascular densities with BCVA. Previous papers of others and ours have reported the decreasing inner retinal microvascular density and alterations of outer retinal sublayer thicknesses in simple high myopia (Table 6), respectively. Based on laser Doppler velocimetry and other techniques, all of which have limitations for measuring retinal capillaries, the density of the retinal vessels in simple high myopia was reported to be reduced.[13,14] With the advancement in high resolution OCTA, our previous study corroborated the reduced density of the retinal microvasculature network in simple high myopia.[11] Further, Mo et al. reported a significant decrease in retinal vessel density in pathological myopia, although they did not use the image magnification correction methods.[15] Changes in the outer retinal sublayer thicknesses, such as thickening of the OPL

Table 5. Linear Regression Based on Best Corrected Visual Acuity Outcome for All High Myopia Patients

	Univariate Regression Analysis			Multivariate Regression Analysis		
Parameters	**Unstandardized Coefficients**	**Standardized Coefficients**	***P* Value**	**Unstandardized Coefficients**	**Standardized Coefficients**	***P* Value**
AL, mm	0.034	0.422	**<0.001**	–	–	–
Sex, male	0.067	0.028	**0.021**	–	–	–
OPL, µm	0.009	0.288	**0.016**	–	–	–
HFL + ONL, µm	−0.002	−0.223	0.063	–	–	–
MEZ, µm	−0.028	−0.528	**<0.001**	−0.014	−0.278	**0.010**
OS, µm	0.006	0.230	0.055	–	–	–
IZ + RPE, µm	−0.013	−0.502	**<0.001**	–	–	–
Choroid, µm	−0.001	−0.542	**<0.001**	−0.001	−0.297	**0.008**
SRCP, %	−0.022	−0.398	**0.001**	–	–	–
DRCP, %	−0.012	−0.524	**<0.001**	−0.008	−0.329	**0.003**

R^2, 0.456 for the multivariate regression analysis.

Table 6. Summary of Previous Studies on Outer Retinal Sublayers and Retinal Microvascular Changes in Myopic Eyes

Study	**Age, Years**	**SE, Diopter**	**AL, mm**	**Fundus Structural and Microvascular Changes**
Current study	Simple high myopia: 32.4 ± 9.1	Simple high myopia: −10.31 ± 2.67	Simple high myopia: 27.56 ± 1.16	Thickness of HFL + ONL, MEZ, OS and IZ+RPE: Simple high myopia > Pathological myopia
	Pathological myopia: 37.7 ± 11.4	Pathological myopia: −13.88 ± 3.25	Pathological myopia: 29.21 ± 1.24	DRCP: Simple high myopia > Pathological myopia
Solmaz et al.[31]	HM: 31.9 ± 9.9 EM: 32.5 ± 9.3	HM: −6.78 ± 2.00 EM: 0.26 ± 1.17	HM: 26.89 ± 0.60 EM: 23.57 ± 0.56	Thickness of ONL: HM < EM
Liu et al.[8]	HM: 24.43 ± 3.43 EM: 27.15 ± 4.51	HM: −7.85 ± 1.37 EM: −0.02 ± 0.39	HM: 26.61 ± 0.97 EM: 23.72 ± 0.69	Thickness of OPL: HM > EM; Thickness of HFL + ONL: HM < EM
Li et al.[32]	HM: 28 ± 5	HM: −6.31 ± 1.23	HM: 26.44 ± 0.97	Retinal Microvascular Network: HM < EM
	EM:30 ± 6	EM: −1.40 ± 1.00	EM:24.13 ± 0.95	
Yang et al.[11]	HM: 26.0 ± 2.7	HM: –8.68 ± 1.87	HM: 27.11 ± 1.27	Retinal Microvascular Network: HM < EM to MM
	EM to MM: 27.4 ± 6.4	EM to MM: –0.77 ± 1.01	EM to MM: 23.84 ± 0.82	
Mo et al.[15]	Simple high myopia: 33.3 ± 15.0	Simple high myopia: –6.90 ± 1.23	Simple high myopia: 25.93 ± 0.58	Retinal microvascular density: Pathological myopia < Simple high myopia < EM
	Pathological myopia: 38.0 ± 11.7	Pathological myopia: −15.22 ± 3.79	Pathological myopia: 29.55 ± 1.73	
	EM: 38.3 ± 13.1	EM: 0.07 ± 0.35	EM: 23.19 ± 0.58	

HM, high myopia; EM, emmetropia; MM, moderate myopia.

and thinning of the ONL, have been reported previously.[2,8] Our recent studies also demonstrated that the degeneration of photoreceptors played an important role in visual dysfunction.[2,3] In the current study, we further investigated the associations between retinal structure and the microvasculature and the combined effects on visual impairment. Importantly, we found that loss of the inner retinal macular microvasculature was correlated with the thinning of outer retina structure and visual function in pathological myopia.

For the inner retinal microvasculature, the density of only the DRCP decreased in the pathological myopia eyes when compared to simple high myopia eyes. This indicates that the deep retinal macular flow density decreased more drastically than did the superficial retinal flow in pathological myopia. The superficial retinal microvasculature is located mainly near the large retinal vessels that are in the retinal nerve fiber layer, so its density might be less sensitive to alterations during pathological changes associated with high myopia.[16,17] On the other hand, the DRCP is the most vulnerable vascular bed and hardest to be repaired, which may explain the serious loss of density in pathological myopia.[18–20] Moreover, the capillaries in the deep macula are important for the transport of the oxygen to the retinal structures, and the capillary flow density is greater in the deep retinal macula than in the superficial retinal macula in most conditions.[21,22] Therefore, the deep retinal microvasculature may be the most essential perfusion plexus in the retina.[23–25]

Importantly, our current results demonstrated significant correlations between the DRCP and the outer retinal sublayer thicknesses in pathological myopia but not in simple high myopia. In normal nonmyopic eyes, the DRCP contributes only 10% to 15% of the oxygen demand of the outer retina.[6] Considering that the visual function remained

normal and stable in simple high myopia, although there was a decline in choroidal thickness, we hypothesize that the diffusion of oxygen from the choroid is sufficient to meet the demands of the outer retinal sublayers and prevent visual impairment. Therefore, in simple high myopia, the correlation between thicknesses of the outer retinal sublayers and the inner retinal capillary plexus density did not reach the statistical significance.

To our best knowledge, this is the first report of the relationship between the thicknesses of the outer retinal sublayers and the density of the DRCP in pathological myopia. The cause-and-effect relationship between DRCP density reducing and pathological changes in the outer retinal sublayers is unclear. One explanation of our current results might be that the degeneration of the outer retina, mainly the photoreceptors, results in the lower oxygen demand during the maculopathy. In simple high myopia, retinal blood flow velocity and dynamic functions, such as dilation of retinal arterial and venous vessels remain unchanged even when the thickness of the outer retinal sublayers are diminished.[2,13,26] Thus, the thinning of the DRCP might be secondary to the thinning of the outer retinal sublayers.

On the other hand, our current study of multiple regression analysis showed that reduced DRCP density was the most important factor related to visual loss. In our previous and current studies, we found that the MEZ was essential for visual function and the MEZ thickness was correlated to the DRCP density, even after adjustment for axial length. This would indicate that the continued reduced DRCP density might be related to the reduced oxygen and nutrient availability for the outer retinal sublayers, thus further associated with the loss of retinal outer sublayer structure and function. During the degeneration of choroidal perfusion in pathological myopia, the DRCP might become more significant in meeting the greater oxygen demand of the outer retina. The failure of DRCP autoregulation in pathological myopia may compromise this potential contribution and be associated with the impaired metabolism of the outer retina. Then, degeneration of both the inner retinal microvasculature and the choroid would be related to the ischemia of the outer retinal sublayers. The resulting hypoxia is likely to fail in maintaining normal visual function within the damaged macula.

Moreover, the DRCP is important for oxygen and nutritional support of the synaptic connections of photoreceptors with the horizontal and bipolar cells.[27,28] Loss of the horizontal and bipolar cells would have significant effects on photoreceptor survival and function, resulting in visual impairment.[27] Therefore, hypoperfusion of the deep retinal microvasculature may be related to the nutritional deficiency in the cells around the synaptic connections and photoreceptors. The loss of oxygen and nutritional resources would be associated with the structural impairment, and then result in the visual impairment in pathological myopia. This may give us clues to develop a strategy of visual protection for patients with pathological myopia.

In our current result, the OS was thicker in simple high myopia and pathological myopia when compared to the control group. The OS thickening was also found in the myopia animal model.[29] Interestingly, the elongation of the OS layer was found to correlate with the decreasing of DRCP density. This may be due to the anteroposterior tractional force on the outer retina and tangential stretching force on the inner retina resulting from the myopic global expansion, which may also lead to the stretching of the DRCP.

We acknowledge some limitations in this study. First, to draw more reliable conclusions about the inner retinal microvasculature and its association with the outer retina and visual function in pathological myopia, we need to include a larger sample size of subjects in the future. Second, when measuring the inner retinal microvasculature, we excluded the FAZ while assuming it was the same size for all subjects. The size of the FAZ might vary among subjects, although it shows most subjects' FAZ was smaller than the circle with the 0.8-mm diameter.[30] When calculated, the inner retinal microvascular density with the exclusion of the FAZ with a 0.8-mm diameter circle, we could avoid the confusing factor that the decreased microvascular density is just due to the enlarged FAZ to some extent. In further research with more subjects, we intend to update our software and do a more thorough analysis of the FAZ size. The pathological myopia consisted of more than four times more women than men, whereas the simple high myopia has less than twice more women than men in our study. The big different ration of women and men among the three groups might influence the results, although the *P* values were not significant. In our future study, we would try to match the women and men with similar proportions in different groups. Moreover, we did the survey about medical history and current symptoms for all subjects, including the color vision. All subjects denied the difficulty in discriminating colors, whereas their color vision was not examined by the professional examiner in this experiment. We had excluded most suspected subjects with high myopia with other diseases, but finer examination should be done to exclude the subjects in the future. Finally, this was a cross-sectional study, but a longitudinal study is required to confirm the alterations of the outer retinal sublayers and retinal microvasculature during the natural progression of pathological myopia.

In conclusion, we measured alterations of the inner retinal microvasculature and outer retinal sublayer thicknesses in simple high myopia and pathological myopia. In the pathological myopia eyes, changes in the deep retinal microvasculature density were correlated with the thickness of the outer retinal sublayers and visual acuity. The changes in thickness of the choroid, DRCP density, and the outer retinal sublayer thicknesses could all play significant roles in the mechanism of the visual impairment characteristic of pathological myopia. Thus, the inter-relationships among the outer retinal sublayers, DRCP, and visual functions in pathological myopia warrant further study.

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