Although intraocular pressure (IOP)-related stress and strain play a central role in glaucoma, a potential pathogenic role of the microvasculature and ocular blood flow in the development and progression of glaucomatous optic neuropathy has long been recognized. The deep-layer (i.e., below the retina) microvasculature within the parapapillary area is of particular interest because it is downstream from the short posterior ciliary artery, which also perfuses the deep optic nerve head (ONH). However, characterization of the parapapillary deep-layer microvasculature has been limited by a lack of methods to clinically visualize it.

Optical coherence tomography angiography (OCTA) is a new imaging technique that enables visualization of the vasculature of different layers. Studies using OCTA reported a regional microvasculature dropout (MvD) in the parapapillary deep layer in glaucoma patients. The MvD was observed as a complete sectoral loss of the choriocapillaris using en face choroidal layer vessel density maps. However, the biological and/or functional nature of MvD is largely unknown. Because no vascular signal is observed at the MvD, it is expected that the choroid is either absent (i.e., zone) or severely atrophic at the location where MvD is observed. Alternatively, it is possible that it represents a compromise of the existing microvasculature (i.e., vasospasm or obliteration). Finally, it is possible that MvD is simply an artifact of OCTA.

We hypothesized that characterizing the microstructure of the MvD should facilitate differentiation between these possibilities and allow further insight into this intriguing phenomenon. Therefore, we performed this study to evaluate the microstructure of the parapapillary area in which the MvD was identified by OCTA using swept-source OCT.

**METHODS**

This prospective study characterized the peripapillary circulation using OCTA in consecutive primary open-angle glaucoma (POAG) patients who were enrolled in the Investigating Glaucoma Progression Study, an ongoing prospective study of glaucoma patients at the Glaucoma Clinic of Seoul National
Baseline Characteristics

<table>
<thead>
<tr>
<th>Eyes With β-Zone (A)</th>
<th>Eyes With γ-Zone (B)</th>
<th>Eyes With β+γ-Zone (C)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 72</td>
<td>n = 57</td>
<td>n = 59</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>65.8 ± 8.9</td>
<td>44.9 ± 11.3</td>
<td>46.9 ± 12.0</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>33/39</td>
<td>35/22</td>
<td>26/33</td>
</tr>
<tr>
<td>Untreated IOP, mm Hg</td>
<td>16.3 ± 4.3</td>
<td>16.0 ± 3.8</td>
<td>16.3 ± 5.3</td>
</tr>
<tr>
<td>IOP at OCTA, mm Hg</td>
<td>11.9 ± 3.7</td>
<td>12.1 ± 2.5</td>
<td>12.0 ± 2.5</td>
</tr>
<tr>
<td>Spherical error, D</td>
<td>0.69 ± 1.54</td>
<td>-6.22 ± 2.88</td>
<td>-4.49 ± 3.95</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>23.46 ± 0.72</td>
<td>26.43 ± 1.62</td>
<td>26.05 ± 1.49</td>
</tr>
<tr>
<td>Central corneal thickness, µm</td>
<td>552.6 ± 40.4</td>
<td>542.7 ± 51.1</td>
<td>546.8 ± 39.9</td>
</tr>
<tr>
<td>Global RNFL thickness, µm</td>
<td>70.3 ± 11.8</td>
<td>66.9 ± 13.0</td>
<td>65.8 ± 10.7</td>
</tr>
<tr>
<td>Visual field mean deviation, dB</td>
<td>-9.52 ± 6.12</td>
<td>-8.09 ± 6.70</td>
<td>-7.85 ± 6.92</td>
</tr>
</tbody>
</table>

Values with statistical significance are shown in bold. Data are shown in mean ± SD, unless otherwise specified. RNFL, retinal nerve fiber layer.

University Bundang Hospital Glaucoma Clinic. Written informed consent to participate was obtained from all patients. The study protocol was approved by the Institutional Review Board of Seoul National University Bundang Hospital, and it adhered to the tenets of the Declaration of Helsinki.

All patients underwent a comprehensive ophthalmic examination that included best-corrected visual acuity (BCVA), Goldmann applanation tonometry, a refraction test, slit-lamp biomicroscopy, gonioscopy, stereo disc photography, red-free fundus photography (EOS D60 digital camera; Canon, Utsunomiya, Japan), a central corneal thickness measurement (Orbscan II; Bausch & Lomb Surgical, Rochester, NY, USA), an axial length measurement (IOLMaster Version 5; Carl Zeiss Meditec, Dublin, CA, USA), standard automated perimetry (Humphrey Field Analyzer II 750, 24-2 Swedish interactive threshold algorithm; Carl Zeiss Meditec), spectral-domain OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany), and swept-source OCT and OCTA (DRI OCT Triton; Topcon, Tokyo, Japan).

Primary open-angle glaucoma was defined as the presence of an open iridocorneal angle, signs of glucomatous optic nerve damage (i.e., neuroretinal rim thinning, notching, or a retinal nerve fiber layer defect), and a glucomatous visual field (VF) defect. A glucomatous VF defect was defined as a defect conforming with one or more of the following criteria: (1) outside normal limits on a Glaucoma Hemifield Test; (2) three abnormal points with a P < 5% probability of being normal, and one abnormal point with a P < 1% probability by pattern deviation; or (3) a pattern standard deviation with a P < 5% probability confirmed on two consecutive reliable tests (a fixation loss rate of ≤20% and false-positive and false-negative error rates of ≤25%). The normal controls had an IOP of ≤21 mm Hg, no history of increased IOP, an optic disc with a normal appearance, and a normal VF.

The eyes were required to have a record of untreated IOP which was measured prior to the initiation of ocular hypotensive treatment or identified in the referral notes. In patients with an untreated IOP ≤21 mm Hg, the diurnal variation was measured during office hours (9 AM–5 PM). In patients who were undergoing treatment with ocular hypotensive medication at the time of the initial visit, the diurnal variation was measured after a 4-week washout period. The exclusion criteria were eyes with a BCVA worse than 20/40, a spherical equivalent of ≤-6.0 diopters [D] or ≥7.0 D, a cylinder correction of ≤-3.0 D or ≥3.0 D, a history of intraocular surgery with the exception of uneventful cataract surgery or trabeculectomy, or retinal or neurologic diseases.

When both eyes were eligible, one eye was randomly selected for inclusion in the study.

**Optical Coherence Tomography Angiography**

The optic nerve and peripapillary areas were imaged using a commercially available swept-source OCTA device (DRI OCT Triton, Topcon), with a central wavelength of 1050 nm, an acquisition speed of 100,000 A-scans per second, and an axial and transversal resolution of 7 and 20 µm in tissue, respectively. Scans were taken from 4.5 × 4.5-mm cubes with each cube consisting of 320 clusters of four repeated B-scans centered on the optic disc.

The deep-layer microvasculature in the peripapillary area was evaluated in the en face images of the peripapillary deep layer generated based on the automated layer segmentation performed by the OCT instrument software. The en face images of the deep layer were derived from an en face slab, extending from Bruch’s membrane (BM) to 390 µm below the BM (a default value of the DRI OCT Triton), which was sufficient to include the full thickness of the choroid and inner sclera.

The MvD was defined as a focal sectoral capillary dropout without any visible microvascular network identified in the deep-layer en face image. When circumferential width of the area with capillary dropout looked more than two times greater than the width of visible juxtapapillary microvessels, it was considered a disruption of the microvascular network, and an MvD was defined. Two independent observers (EJL and SHL) identified MvDs while being blinded to the clinical information of the patients. Disagreements between these two observers were resolved by a third adjudicator (FWK). Only
eyes with MvDs were included in the study. When the quality of the OCTA image was poor (i.e., blurred images that hampered the delineation of the MvD), the eye was excluded from the analyses.

The location and extent of the MvD were determined by recording the clock hours and the number of clock hours involving the MvD. The clock-hour meridians were determined based on the locations where the radial optic disc scanning was obtained using swept-source OCT. This was performed by superimposing and manually aligning the OCTA images on the fundus image provided in the swept-source OCT, using commercial software (Photoshop CC; Adobe Systems, Mountain View, CA, USA).

OCT Scanning of the Peripapillary Area

Swept-source OCT was performed using the DRI-OCT Triton (Topcon) to assess the structure of the peripapillary deep layer and to measure the juxtapapillary choroidal thickness (JPCT). This system used a light source consisting of a wavelength sweeping laser centered at 1050 nm, with a repetition rate of 100,000 Hz, yielding an axial resolution of 8 μm in tissue. An increased light transmission of this device allows better visualization of deep-layer tissues including the choroid and sclera.

Images were obtained using a 6-mm, 12 radial line scan, centered on the optic disc. Sixteen single images were registered and averaged for each line scan. To be included in the analyses, all images had to have an image quality score \( \geq 45 \), according to the manufacturer's recommendation.

It has been demonstrated that MvD is correlated with \( \beta \) parapapillary atrophy (βPPA), which can be divided into an area with BM and underlying choroid (i.e., β-zone), and an area devoid of either tissues (i.e., γ-zone). Because no choroidal tissue existed in the γ-zone, the JPCT was measured only in eyes in which the MvD was associated with the β-zone.

The average JPCT at each of 12 clock-hour meridians was measured using six radial scans. To accomplish this measure-
ment, the area of the choroidal tissue within 500 μm from the BM termination point was first measured, then divided by 500 μm using ImageJ software (National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/; in the public domain). The measurements were performed by independent observers (EJL and SHL) who were masked to the patient’s information. The average of two measurements from each observer was used for analyses.

For comparison of the JPCTs, 72 eyes without MvD and having a β-zone were included as a control group after matching the age, untreated IOP, and VF mean deviation with the study group.

Data Analysis

The interobserver agreement regarding confirmation of the presence of the MvD and measurements of the JPCT was determined using kappa statistics (i.e., κ value) and intraclass correlation coefficients, respectively. The comparison between groups was performed using Student’s t-test. All statistical analyses were performed using SPSS statistical software for Windows, version 19.0 (SPSS, Chicago, IL, USA). The data were expressed as the mean ± standard deviation (SD) except when stated otherwise, and the cutoff for statistical significance was set at $P < 0.05$.

RESULTS

Three hundred fifty POAG eyes that underwent an OCTA examination were initially included. Of these eyes, 15 were excluded due to a poor-quality OCTA image. Of the remaining 335 eyes, an MvD was observed in 188 eyes (56.1%). Of these, 172 eyes had a single MvD, and 16 eyes had two separate MvDs, resulting in a total of 204 MvDs. The number of clock-hour sectors involving each MvD and the frequency of MvD involvement in each clock-hour sector are shown in Figure 1. There was excellent interobserver agreement regarding the detection of the MvD (κ = 0.958).

All eyes with MvDs had a βPPA. The MvD was found in either the β-zone, γ-zone, or a mixture of the β- and contiguous γ-zones in 72, 57, and 59 eyes, respectively. Eyes with MvDs were divided according to the type of βPPA in the area with
MvD: $\beta$-zone group, $\gamma$-zone group, and $\beta+\gamma$-zone group. A comparison of the baseline characteristics between the different $\beta$PPA groups is given in the Table.

**Underlying Microstructure Shown by Swept-Source OCT at the Area of the Parapapillary Deep-Layer MvD**

In the eyes with a $\beta$-zone, the choroid and sclera in the area of the PPA had a higher reflectivity, regardless of their association with an MvD. Choroidal tissue of noticeable thickness was observed in all eyes in the area of the MvD (Fig. 2). In eyes with a $\gamma$-zone, the PPA area consisted of border tissue of Elschnig that does not have the choroidal tissue. Microvasculature was clearly seen in deep-layer OCTA images in the area of the $\gamma$-zone when the MvD was absent. No visible difference was detected in the microstructure between the $\gamma$-zone with or without the MvD (Fig. 3). Similar findings were observed in eyes where the MvD was associated with both the $\beta$- and $\gamma$-zones; a choroid of noticeable thickness was observed in the $\beta$-zone, and no distinguishable feature was found in the $\gamma$-zone from eyes without the MvD (Fig. 4).

**Comparison of the Juxtapapillary Choroidal Thickness**

Excellent interobserver agreement was obtained in each clock-hour sector (intraclass correlation coefficients, 0.945–0.996). There was no significant difference in the JPCT at any clock-hour location between eyes with the $\beta$-zone having an MvD and those not having an MvD (Fig. 5).

**DISCUSSION**

We used swept-source OCT to characterize the microstructure of the parapapillary deep-layer tissue in the area of the MvD, and found that the location of the MvD involved either the $\beta$-zone, $\gamma$-zone, or a mixture of both. To our knowledge, this is the first study that characterized the microstructure of deep-layer tissue at the location of parapapillary MvDs.

The choroid comprises three main vascular layers involving the choriocapillaris, Sattler’s layer, and Haller’s layer, in order from the inner to the outer choroid. Sattler’s layer contains medium-sized blood vessels that supply the choriocapillaris, and Haller’s layer contains large vessels. Because the MvD was observed with the total absence of a vasculature, we expected that the choroidal tissue was severely atrophic without detectable vessels at the location of the MvD. However, this
was not the case in eyes with MvDs that were associated with the β-zone; there was observable choroidal tissue at the location of the MvD. Furthermore, the JPCT in eyes with MvD was not smaller than in those without MvD.

It is possible that the absence of the vasculature within the MvD is partly due to unknown reasons involving the invisibility of large vessels by OCTA (e.g., pulsatile flow in which there was insufficient flow to be detected in one interval between repeated B-scans15). If signals from large vessels cannot be detected by OCTA, an MvD would be detected when the choriocapillaris was atrophic, regardless of whether the large vessels in the underlying layer were intact. However, large vessels were clearly visualized when en face OCTA images were generated for a thin slab that included only the outer choroid (Fig. 6). Thus, the MvD seen in the en face images of the full-thickness choroid may indicate an absence of blood flow within the choriocapillaris and within the large choroidal vessels.

Although the reasons are unknown, MvD was observed despite the presence of choroidal tissue. One possible hypothesis is that the vessels supplying or draining the choriocapillaris were partially or completely obliterated. The choriocapillaris is a meshwork of densely packed, interconnected vessels forming characteristic meshlike structures that are organized in a lobular structure.14 The choriocapillaris lobule is supplied by feeding arterioles located in the center of the lobules,15 and drained by peripheral venules surrounding the lobules. If feeding or draining vessels were obliterated, a perfusion defect would develop in the involved choriocapillaris lobule. This would be seen as a sectoral MvD. Another possibility is that the MvD detected by OCTA was an artifact that was not indicative of a true vascular compromise. Optical coherence tomography angiography is subject to various artifacts,13 including image artifacts, shadowing, or segmentation errors that may result in a false-negative flow signal, which could be misinterpreted as a vascular defect.13 A comparison with indocyanine green angiography, which can visualize the perfusion in the choroid, would be able to clarify whether the MvD represents a real perfusion defect.

The γ-zone does not contain the choroid. However, vascular signals were clearly identified in the γ-zone in the deep-layer (i.e., excluding the retinal layer) en face OCTA images. The vessels could be those within the scleral flange. In the present study, a regional MvD was also found within the γ-zone. The γ-zone develops as a result of scleral stretching, driven by axial elongation in myopic eyes.11,16 In this process, tensile stress may be exerted on the vessels within the sclera. We speculate that long-standing tensile stress may disrupt the structural integrity of some vessels, eventually leading to an MvD. Alternatively, it is possible that the MvD in the γ-zone is an artifact.

When the MvD area was seen within the β-zone, the choroidal tissue showed hyperreflectivity compared with the adjacent choroid. One may consider that the hyperreflectivity suggests that there is a structural change in the choroidal tissue at the area of the β-zone (i.e., atrophy). However, hyperreflectivity was also observed in the choroid in the area of the β-zone without the MvD. In addition, the sclera below the β-zone also appeared hyperreflective (Fig. 2E, arrow). Thus, the hyperreflectivity may be the result of increased light transmission through the atrophic retinal pigment epithelium at the β-zone.

There was a subset of eyes in which the MvD simultaneously involved both the β- and γ-zones. It is unknown whether the MvD developed independently in the two zones or developed first in one zone, followed by MvD induction in the other zone. A longitudinal study is required to answer this question.

This study included a control group without an MvD for comparison of the JPCT with the eyes having MvD. The control group was matched with the study group for age, untreated IOP, and VF mean deviation but not for axial length. Previous studies demonstrated that a longer axial length was associated with a thinner macular17,18 and peripapillary choroid (i.e., atrophy). However, the choroidal thickness of the juxtapapillary area might have been less affected. Based on our previous result, we did not match axial length between groups. Nonetheless, the axial length did not differ between the groups (Supplementary Table S1). Taken together, the effect of axial length on the choroidal thickness between the groups, if any, may be negligible.

A limitation of the OCTA is that flow projection artifacts by retinal vasculature or the signal of the choroidal large vessels could hamper a precise evaluation of the MvD.23,24 The possibility that MvD would be difficult to detect in the area with retinal vasculature and/or choroidal large vessels should be considered when interpreting the OCTA.

In conclusion, OCTA-detected MvDs were found in both the β- and γ-zones. When they were found in the β-zone, they were not necessarily associated with obvious thinning of the choroidal tissue, indicating that the MvD resulted from the closure of vessels supplying the existing choroidal lobules.
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References