Optical Coherence Tomography Angiography Reveals Spatial Bias of Macular Capillary Dropout in Diabetic Retinopathy

Yoshihiro Kaizu,[1] Shintaro Nakao,[1] Shigeo Yoshida,[1] Takehito Hayami,[2] Mitsuru Arima,[1] Muneo Yamaguchi,[1] Iori Wada,[1] Toshio Hisatomi,[1] Yasuhiro Ikeda,[1] Tatsuro Ishibashi,[1] and Koh-Hei Sonoda[1]

[1]Department of Ophthalmology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[2]Department of Intelligent Mechanical Systems, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan

Correspondence: Shintaro Nakao, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812–8582, Japan; snakao@med.kyushu-u.ac.jp.

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Purpose. Our purpose is to evaluate the spatial bias of macular capillary dropout accompanying diabetic retinopathy (DR) using optical coherence tomography angiography (OCTA).

Methods. This study included 47 patients with diabetes and 29 healthy individuals who underwent OCTA. Retinal capillary flow density (FD) of 2.6 × 2.6 or 5.2 × 5.2 mm foveal area as well as the four divided areas (superior, inferior, temporal, nasal) without a foveal avascular zone (FAZ) at the superficial capillary plexus and deep capillary plexus (DCP) were measured respectively using ImageJ and NI Vision. Spatial biases of FD (orientation bias ratio and hierarchical bias ratio) and the correlation between FAZ and FD were examined.

Results. OCTA showed focal capillary dropout in DR patients. The orientation bias of FD was significantly higher in NPDR compared to NDR in the DCP ($P = 0.03$). The hierarchical bias of FD was significantly shifted to a DCP dominance with progression of DR ($P < 0.01$). In addition, the FD and FAZ area were significantly inversely correlated in both plexus in DR patients but not in healthy subjects ($P < 0.01$).

Conclusions. Area-divided OCTA quantification shows the appearance of spatial biases of macular capillary dropout with the onset of DR, suggesting that DR-related macular capillary dropout occurs locally and randomly. Future studies are necessary to determine the clinical relevance of the spatial pattern of capillary dropout in DR.

Keywords: vascular density, vessel density, nonflow area, nonperfusion area, retinal capillary dropout

Diabetic retinopathy (DR) is a major cause of blindness among adults of working age worldwide.[1] The pathogenesis of DR is due to high glucose-related retinal microvascular complications such as microthrombosis.[2] Preclinical studies have shown that retinal capillary dropout could be induced by various mechanisms (e.g., leukostasis or platelet aggregation) during the progression of DR.[3,4] Depending on the capillary dropout, the lesion further leads to vitreous and neuronal disorders resulting in visual acuity loss.[5,6] Therefore, for the prevention of blindness, a more detailed and accurate quantitative understanding of capillary dropout in DR patients is required.

Fluorescein angiography (FA) is an established method for observing fine details of retinal microvascular damage in DR. However, the measurement of this capillary dropout is not quantitative and spatial detection is also impossible since FA fails to detect a significant fraction of capillaries due to poor depth sectioning capacity and sensitivity to the angiogram quality.[7] For this reason, the spatial pattern of capillary dropout has not been examined in DR patients.

Optical coherence tomography angiography (OCTA) is a noninvasive imaging device used to examine the retinal vasculature and its hierarchical structures without any contrast agent injection.[8,9] Various reports have shown the utility of OCTA in imaging retinal vascular damage in DR patients.[10–16] A pilot study first showed pathologic vascular changes in DR with OCTA.[13] Two groups independently reported an enlargement of the foveal avascular zone (FAZ) in DR using OCTA.[12,14] Several groups reported that quantitative vascular flow density (FD) decreased in both the superficial capillary plexus (SCP) and the deep capillary plexus (DCP) in DR.[10] Most studies by OCTA investigated vascular FD including FAZ, where FAZ enlargement could cause a decline of FD in a regulated imaging frame (e.g., 3 × 3 mm).

A previous histologic study by Kern et al.[17] using a diabetic animal model reported that vascular disorders could be significantly more prevalent in the superior temporal retina than in the inferior nasal quadrant of the retina. Furthermore, Tang et al.[18] also showed that diabetic vascular abnormalities occurred significantly more frequently in the temporal retina than in the nasal retina with the trypsin-digest method in diabetic human donor eyes. A recent ultrawide-field imaging study using Optos devices (Dunfermline, UK) also revealed that diabetic vascular abnormalities were more frequent in the

temporal fields compared with the nasal fields.[19] However, the spatial pattern of diabetic vascular abnormalities has not been examined using OCTA. Furthermore, the hierarchical bias of capillary dropout has not been examined in DR. In this study, the spatial pattern of macular capillary dropout accompanying DR using OCTA was investigated.

Methods

This study was approved by the Institutional Ethics Committees of the Kyushu University Hospital, and was performed in accordance with the tenets of the Declaration of Helsinki.

Patient Population

This was a retrospective, observational, cross-sectional study. We retrospectively evaluated 64 eyes of 47 patients with type 1 or type 2 diabetes mellitus (DM) and 29 eyes of 29 healthy individuals. All patients and control individuals underwent OCTA at Kyushu University Hospital between November 2014 and June 2017. The healthy individuals who were recruited had no history of prior ocular or systemic disease. Exclusion criteria included the presence of macular edema. Eyes with poor quality OCT images due to cataract, vitreous hemorrhage or poor fixation were also excluded.

Optical Coherence Tomography Angiography

All OCTA images were obtained using a commercial imaging device (RTVue XR Avanti; Optovue, Inc., Fremont, CA, USA). This instrument has an A-scan rate of 70,000 scans per second, using a scan light centered at 840 nm with a bandwidth of 45 nm and a tissue resolution of 5 μm axially. Each B-scan contained 216 A-scans. Five consecutive B-scans (M-B frames) were captured at a fixed position before proceeding to the next sampling location. The scanning areas were 3×3 and/or 6×6 mm cubes centered on the fovea, and we obtained retinal microvascular map images of these areas using OCTA. For each scan, superficial and deep layer OCTA images were generated based on the full automatic retinal segmentation performed by the OCT device software. The definitions of the segmentation are as follows. The SCP layer was defined by the top layer being the inner limiting membrane with a 3-μm offset, and the bottom layer was the inner plexiform layer (IPL) with a 15-μm offset. Conversely, the DCP layer was defined by the top layer being the IPL with an offset of 15 μm and the bottom layer was the IPL with an offset of 70 μm. Furthermore, as it was expected that the "angioFLOW" marks written on the lower left of the en face images would affect the quantitative results of FD, every en face 3×3 and 6×6 mm image (superficial and deep layer) was cropped to a 2.6×2.6 and 5.2×5.2 mm square image centered on the fovea, respectively (Fig. 1).

Foveal Avascular Zone Measurement

We defined FAZ as the inside area of the inner boundary of the central capillary ring using en face SCP imaging (Fig. 1A). Quantifying FAZ at the SCP has been reported to be reliable.[20–22] FAZ areas were manually outlined by a single grader using the ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). We used commercial software (NI Vision; National Instruments Corp., Austin, TX, USA) to calculate the outlined areas in pixels and these were converted into square millimeters based on the 606 pixels width of the original 3×3 mm images.

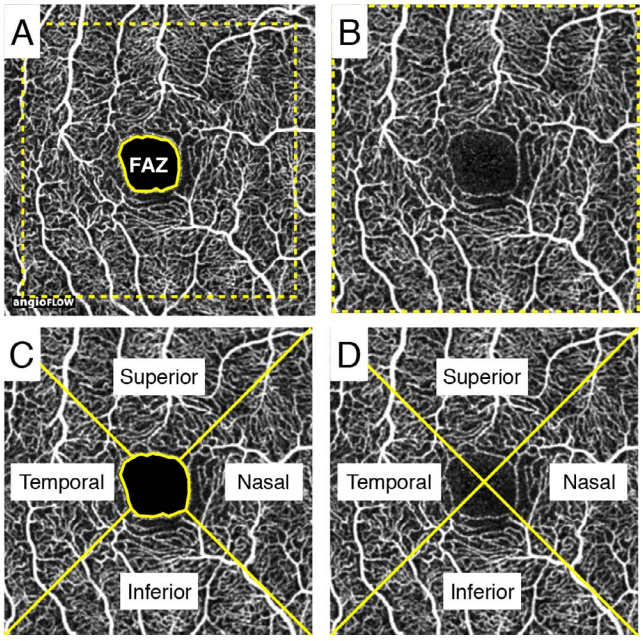

Figure 1. Spatial pattern analysis of OCTA. The OCTA image of a 69-year-old healthy woman at the SCP. (**A**) A 3×3 mm en face OCTA image was cropped to 2.6×2.6 mm using an image editing program (Adobe Systems, Inc.; *yellow dot square*) to avoid influence of "angioFLOW" display on quantification. (**B**) A 2.6×2.6 mm en face OCTA image used for this study. (**C**, **D**) The images were equally divided into four sections (superior, inferior, temoral, nasal). Each area was measured (**C**) without or (**D**) with FAZ using commercial equipment (National Instruments Corp.).

Flow Density Measurement

After cropping 3×3 or 6×6 mm en face OCTA image to 2.6×2.6 or 5.2×5.2 mm, respectively, using an image editing program (Photoshop; Adobe Systems, Inc., San Jose, CA, USA; Figs. 1A, 1B), binarization of the image was carried out using NI Vision, and the images were equally divided into four directions (superior, inferior, temporal, nasal). Each area was measured with or without FAZ using commercial software (National Instruments Corp.; Figs. 1C, 1D). The coefficient of variation was used to measure within-day reproducibility. Five normal subjects underwent three imaging sessions in a single visit. The κ coefficient was 0.848 (95% confidence interval [CI], 0.788–0.893) for the FD measurement as defined above.

Estimation of Orientation Bias of Capillary Dropout

The orientation bias ratio (minimum FD/maximum FD) was calculated for each case from the maximum and minimum values of the FD of the image in four directions.

Estimation of Hierarchical Bias of Capillary Dropout

The ratio of SCP and DCP at 2.6×2.6 and 5.2×5.2 mm was calculated in each case. The ratio of DCP FD/SCP FD was calculated as the hierarchical ratio.

Statistical Analysis

All data were expressed as mean (SD). Statistical analyses were performed using commercial software (JMP Pro 12.2.0; SAS Institute, Cary, NC, USA). Data that were not normally

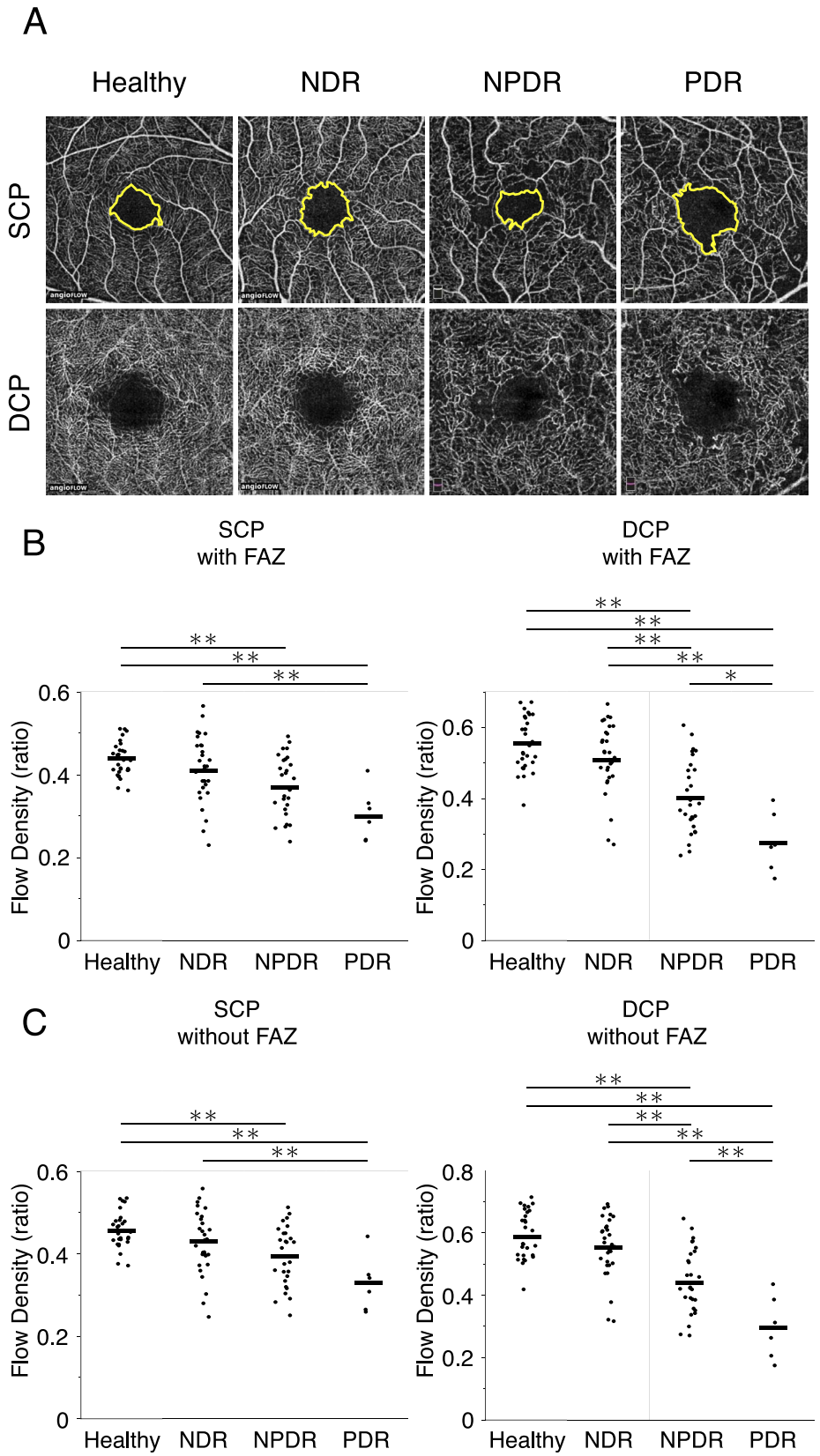

Figure 2. Foveal avascular zone and retinal capillary FD of OCTA in diabetic eyes. (**A**) Representative images of OCTA of the SCP and DCP layers in the macular area of a healthy subject (49-year-old woman); no diabetic retinopathy (45-year-old man); nonproliferative DR (69-year-old man, moderate NPDR); and proliferative DR (42-year-old man). (**B**, **C**) Comparison of quantitative retinal capillary FD of SCP and DCP (**B**) with or (**C**) without the FAZ area among the four groups (healthy, NDR, NPDR, and PDR subjects; $n = 29$, 30, 28, and 6, respectively). $^{*}P < 0.05$. $^{**}P < 0.01$, Tukey–Kramer test.

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Table 1. Comparison of FAZ Area (mm^2) Among Four Groups

FAZ	Healthy	NDR	NPDR	PDR*
Area, mm^2	0.33 ± 0.10	0.35 ± 0.12	0.38 ± 0.15	0.51 ± 0.10*

The four groups studied were healthy, NDR, NPDR, and PDR subjects ($n = 29$, 30, 28, and 6, respectively).

* FAZ area in PDR was significantly larger than in healthy subjects (*$P < 0.05$, Tukey–Kramer test).

distributed were analyzed by nonparametric statistics. The significance of the differences was analyzed using the Student's *t*-test or Tukey–Kramer test. The χ^2 test was used to compare group percentages derived from independent samples. The relationship between the FD area and FAZ area was examined by Pearson's correlation coefficient analysis using a spreadsheet program (Excel; Microsoft Corp., Redmond, WA, USA). *P* values < 0.05 were considered significant.

Results

This study evaluated 93 eyes of 76 patients. There were 72 right eyes and 21 left eyes. A total of 64 eyes of 47 patients with DM were included in this study. The mean age of the patients with DM was 56.6 ± 15.3 years, and 36 (76.6%) were male and 11 (23.4%) were female. Of the eyes studied, 30 (46.9%) had no diabetic retinopathy (NDR); 28 (43.8%) had nonproliferative DR (NPDR; 11 with mild NPDR, 13 with moderate NPDR, and 4 with severe NPDR); and 6 (9.4%) had proliferative DR (PDR). We included 29 eyes of 29 healthy individuals in this study (mean age: 53.6 ± 19.3 years); and 14 (48.3%) were male and 15 (51.7%) were female. The axial length was 24.2 ± 1.3, 24.3 ± 1.3, 23.9 ± 1.2, and 23.2 ± 0.9 mm in healthy, NDR, NPDR, and PDR individuals, respectively. There was no significant difference in axial length among the four groups.

Retinal Capillary Flow Density with or without FAZ in Diabetic Eyes

First, to examine the influence of the FAZ area on retinal FD, we measured the FD of the SCP and DCP with or without FAZ. The FAZ area (mean ± SD) of healthy, NDR, NPDR, and PDR individuals was 0.33 ± 0.10, 0.35 ± 0.12, 0.38 ± 0.15 and 0.51 ± 0.10 mm^2, respectively. The FAZ area was significantly increased in eyes with PDR but not in eyes with NDR and NPDR when compared with healthy eyes ($P < 0.05$; Fig. 2A; Table 1). The FD of the SCP including FAZ was significantly decreased in NPDR and PDR compared to healthy eyes ($P < 0.01$; Fig. 2B). The FD of DCP including FAZ decreased significantly in NPDR and PDR compared to healthy eyes ($P <$

Table 2. Quantitative Retinal Capillary FD of Four Divided Areas of the SCP Layer

SCP (3 × 3 mm)*	Superior	Inferior	Temporal	Nasal
Healthy subjects	0.46 ± 0.04	0.46 ± 0.04	0.45 ± 0.04	0.46 ± 0.04
NDR	0.43 ± 0.10	0.43 ± 0.09	0.43 ± 0.08	0.44 ± 0.08
NPDR	0.40 ± 0.08	0.39 ± 0.08	0.39 ± 0.07	0.40 ± 0.07
PDR	0.33 ± 0.06	0.33 ± 0.07	0.32 ± 0.06	0.33 ± 0.08

Examined in healthy, NDR, NPDR, and PDR subjects ($n = 29$, 30, 28, and 6, respectively).

* There is no significant difference in FD of SCP in the four regions at any stage.

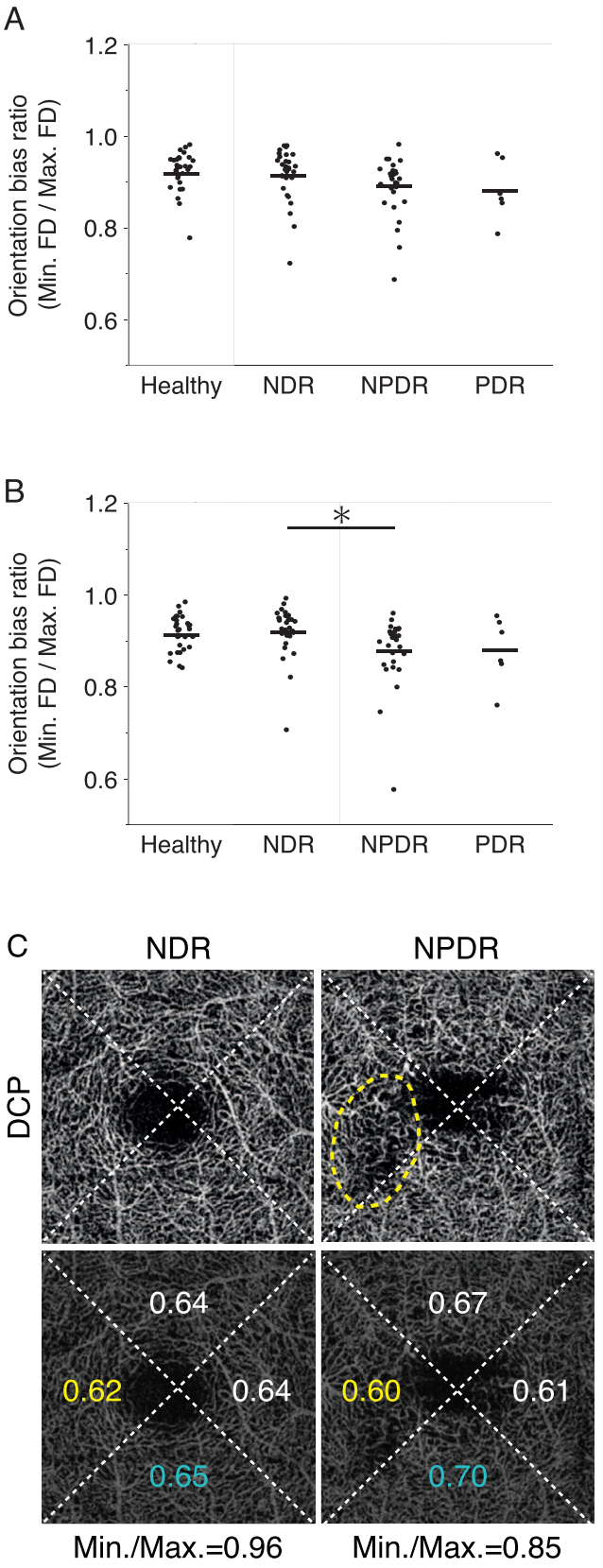

Figure 3. Orientation bias of retinal capillary FD of the (**A**) SCP and (**B**) DCP layers. The ratio of spatial bias was calculated as the minimum FD/maximum FD from the maximum and minimum values of the FD of the image in the four sections in a healthy subject, NDR, NPDR, and PDR ($n =$ 29, 30, 28, and 6, respectively). *$P < 0.05$, Tukey–Kramer test. (**C**) Two representative 3 × 3 mm DCP images of NDR (58-year-old female, right eye) and NPDR (40-year-old male, right eye). *Yellow dot circle* indicates area of local capillary dropout in temporal area of NPDR patients.

Table 3. Quantitative Retinal Capillary FD of Four Divided Areas of the DCP Layer

DCP (3 × 3 mm)*	Superior	Inferior	Temporal	Nasal
Healthy subjects	0.60 ± 0.09	0.60 ± 0.08	0.58 ± 0.08	0.59 ± 0.07
NDR	0.56 ± 0.11	0.56 ± 0.10	0.55 ± 0.09	0.55 ± 0.10
NPDR	0.45 ± 0.11	0.44 ± 0.12	0.44 ± 0.09	0.45 ± 0.10
PDR	0.32 ± 0.09	0.31 ± 0.09	0.31 ± 0.08	0.31 ± 0.10

Examined in healthy, NDR, NPDR, and PDR ($n = 29$, 30, 28, and 6, respectively).

* There is no significant difference in FD of DCP in the four regions at any stage.

0.01; Fig. 2B). In the DCP, the FD was also significantly decreased in the NPDR and PDR groups as compared with the NDR group ($P < 0.01$; Fig. 2B). Furthermore, in the DCP, the FD was significantly decreased in the PDR group as compared with the NPDR group ($P < 0.05$; Fig. 2B). We also measured FD without FAZ. The analysis also showed the same results as the measurement including FAZ ($P < 0.01$; Fig. 2C).

Spatial Pattern of Retinal Capillary Flow Density in Diabetic Eyes

We investigated whether there was a unique spatial pattern for diabetic capillary dropout. Consistent with the previous study, there was no significant difference in the four areas in healthy eyes.[23] In eyes with NDR, NPDR, and PDR, there was no significant difference in FD in the four regions at the SCP (Table 2). The FD in the four regions at the DCP also did not show any significant difference in all groups (Table 3).

Orientation Bias of Retinal Capillary Flow Density in Diabetic Eyes

To examine whether there was spatial bias in capillary dropout at each DR stage, we calculated the spatial bias ratio (minimum FD/maximum FD in four regions) for each case. There was no significant difference between the four groups in the SCP (Fig. 3A). Interestingly, however, the ratio of the NPDR group (0.88 ± 0.07) was significantly lower than one of the NDR groups (0.92 ± 0.05) in the DCP ($P < 0.05$; Fig. 3B). In the NPDR group, the minimum values of FD of DCP were 17.9%, 21.4%, 57.1%, 3.6% in the four divided areas: superior, inferior, temporal, and nasal, respectively. The percentage of the temporal area was the highest among the four groups ($\chi^2 = 62.2$). Statistical significance was evaluated if the value was greater than the level of significance: $\chi^2_{0.05}(3) = 7.81$ (χ^2, $P < 0.01$). There was no significant difference in the spatial bias between NDR and PDR (0.88 ± 0.07) in the DCP. Figure 3C shows two representative DCP images of NDR (58-year-old female, right eye) and NPDR (40-year-old male, right eye). This quantitative analysis showed that local capillary dropout could be observed in NPDR but not NDR patients (Fig. 3C).

Hierarchical Bias of Retinal Capillary Flow Density in Diabetic Eyes

Next, to investigate whether there was hierarchical bias of FD between the SCP and the DCP during the onset and progression of DR, we compared hierarchical bias ratio (DCP FD/SCP FD) among the four groups (Fig. 4). Compared with healthy eyes (1.30 ± 0.13), the ratio was significantly decreased in NPDR (1.13 ± 0.17) and PDR (0.89 ± 0.19; $P < 0.01$). Compared with NDR (1.29 ± 0.12), the ratio was also significantly decreased in NPDR and PDR ($P < 0.01$). Furthermore, the ratio in PDR was significantly lower than in NPDR ($P < 0.01$).

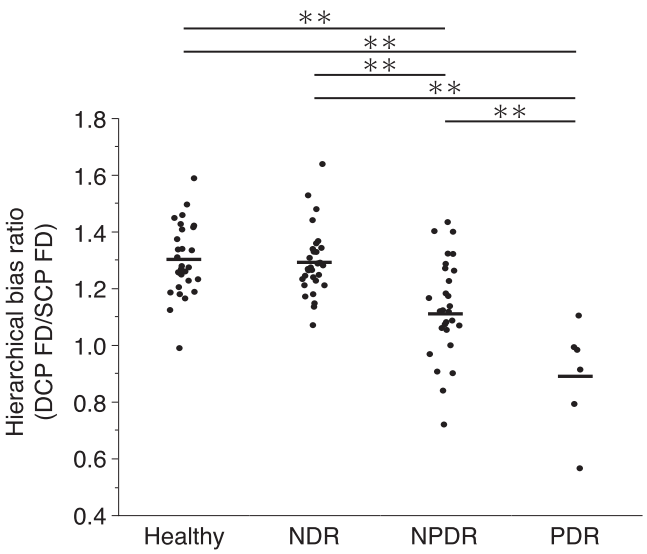

Figure 4. Hierarchical bias of retinal capillary FD in diabetic eyes. The hierarchical bias ratio was calculated as flow density of the DCP per flow density of the SCP in each case in healthy, NDR, NPDR, and PDR subjects ($n = 29$, 30, 28, and 6, respectively). **$P < 0.01$, Tukey–Kramer test.

Spatial Bias of Retinal Capillary Flow Density in 6 × 6 mm Area

To confirm whether the spatial bias observed in 3 × 3 mm OCTA images could also be observed in a wider area, the orientation bias and hierarchical bias ratio of FD for NDR and NPDR were analyzed using 6 × 6 mm en face OCTA images (Fig. 5). Regardless of the inclusion of FAZ, the FD of DCP decreased significantly in NPDR (0.31 ± 0.09 with FAZ, and 0.32 ± 0.09 without FAZ) compared to NDR (0.48 ± 0.12 with FAZ, and 0.48 ± 0.12 without FAZ; $P < 0.01$; Figs. 5A, 5B). On the other hand, no significant difference was observed in the FD of SCP between NDR and NPDR (data not shown). In eyes with NDR and NPDR, there was also no significant difference in FD in the four regions at the SCP (Table 4) as well as the DCP (Table 5) in the 6 × 6 mm. Furthermore, both the orientation bias in DCP and hierarchical bias ratio of FD were significantly decreased in NPDR (0.88 ± 0.04 and 0.93 ± 0.24, respectively) compared to NDR (0.93 ± 0.04 and 1.40 ± 0.22, respectively; $P < 0.01$; Figs. 5C–E). In the NPDR group, the minimum values of FD of DCP were 5.6%, 38.9%, 44.4%, 11.1% in the four divided areas (superior, inferior, temporal, nasal), respectively. The percentage of the temporal area was also the highest among the four groups ($\chi^2 = 45.7$) in 6 × 6 mm OCTA images. Statistical significance was also evaluated if the value was greater than the level of significance: $\chi^2_{0.05}(3) = 7.81$ (χ^2 test, $P < 0.01$).

Correlation Between FAZ Area and Retinal Capillary FD

Finally, we investigated whether the FAZ area correlated with the FD in each capillary plexus. There was a significant inverse correlation between FAZ and FD of both the SCP and the DCP

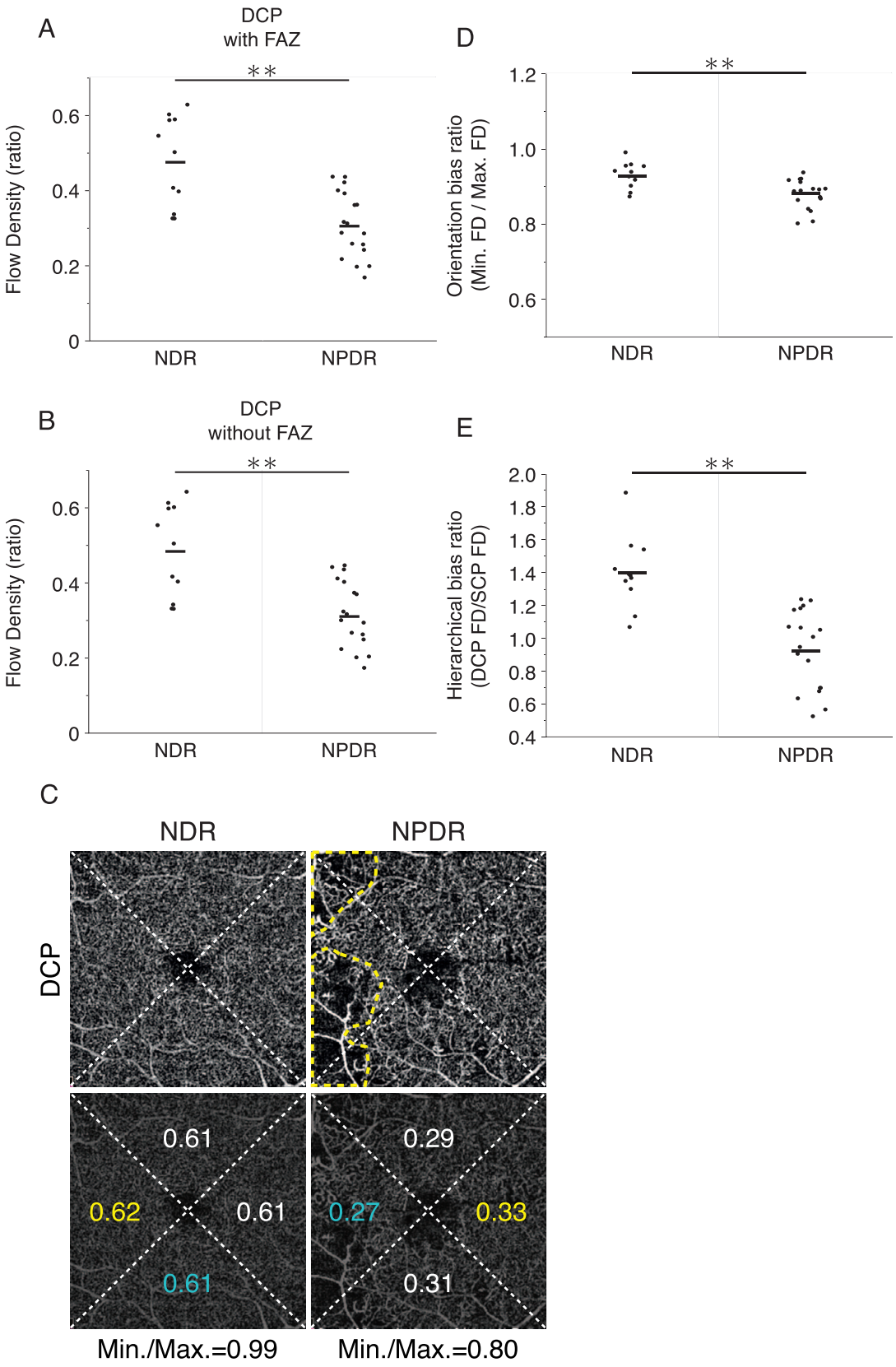

Figure 5. Retinal capillary flow density of 6 × 6 mm OCTA images in diabetic eyes. (**A**, **B**) Comparison of quantitative FD of the DCP (**A**) with or (**B**) without the FAZ area between NDR and NPDR subjects ($n = 11$, 18, respectively). $^{**}P < 0.01$, Student's *t*-test. (**C**) Two representative 6 × 6 mm DCP images of NDR (38-year-old male, right eye) and NPDR (42-year-old male, right eye, severe NPDR). *Yellow dots* indicate areas of local capillary dropout in the temporal area of NPDR patients. (**D**, **E**) Orientation bias of FD of the (**D**) DCP and (**E**) hierarchical bias ratio were compared between NDR and NPDR subjects, respectively ($n = 11$, 18, respectively). $^{**}P < 0.01$, Student's *t*-test.

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Table 4. Quantitative Retinal Capillary FD of Four Divided Areas of the SCP Layer

SCP (6 × 6 mm)*	Superior	Inferior	Temporal	Nasal
NDR	0.34 ± 0.08	0.33 ± 0.07	0.34 ± 0.08	0.35 ± 0.08
NPDR	0.34 ± 0.05	0.34 ± 0.04	0.34 ± 0.05	0.35 ± 0.05

Examined in NDR and NPDR subjects ($n = 11, 18$, respectively).
* There is no significant difference in FD of SCP in the four regions at any stage.

in patients with DM but not healthy subjects ($P < 0.01$; Figs. 6A, 6B).

Discussion

FAZ is a physiologic, capillary-free area in the center of the macula.[24] FA has shown that there is a positive correlation between the size of the FAZ area and severity of DR.[25,26] Recent quantitative studies using OCTA has confirmed this finding.[14,27] Although an OCTA report showed that not only NPDR but also NDR has a larger FAZ area compared with healthy subjects,[14] there was no significant difference between the healthy group and NPDR in this study. Our findings are consistent with another report by Salz et al.[27] This discrepancy could be attributed to the small sample size in these studies as there is large interindividual variability of FAZ size in normal subjects.[28]

Although OCTA studies concerning DR have reported a decrease in retinal capillary FD, most quantitative evaluations have used an FD including the FAZ area.[10,11] As described above,[14,27] an enlarged FAZ is also a finding of capillary dropout in DR. However, it remains unclear whether FAZ expansion and decreased FD are independent or correlated events. Therefore, this study aimed to examine the FD of healthy subjects as well as DM patients separately with and without FAZ. The mean FDs of both capillary plexuses in healthy eyes were significantly greater than in each DR group regardless of whether FAZ was included or not. However, when FAZ was included and not included, the P value was 0.01 and 0.003, respectively, in the comparison of FD of DCP between NPDR and PDR. The quantification of FD without FAZ could thus possibly detect vascular disorders with a higher sensitivity in a regulated imaging frame. Therefore, our study used FD without FAZ to analyze the spatial pattern of capillary dropout.

The FD measurement software was recently installed in the commercial imaging device (Optovue, Inc.) used for this study.[23] According to the Early Treatment Diabetic Retinopathy Study (ETDRS) grid, this software is able to measure the FD in four directions. Some cases, especially in the PDR, detected an FAZ that increased beyond the 1-mm circle of the ETDRS grid. Therefore, the impact of FAZ on FD could not be accurately measured with the installed software. For this reason, we used a measurement method binarized using commercial equipment (National Instruments Corp.) in our study instead.

Table 5. Quantitative Retinal Capillary FD of Four Divided Areas of the DCP Layer

DCP (6 × 6 mm)*	Superior	Inferior	Temporal	Nasal
NDR	0.49 ± 0.12	0.47 ± 0.13	0.48 ± 0.12	0.49 ± 0.13
NPDR	0.32 ± 0.10	0.31 ± 0.09	0.31 ± 0.09	0.32 ± 0.09

Examined in NDR and NPDR subjects ($n = 11, 18$, respectively).
* There is no significant difference in FD of DCP in the four regions at any stage.

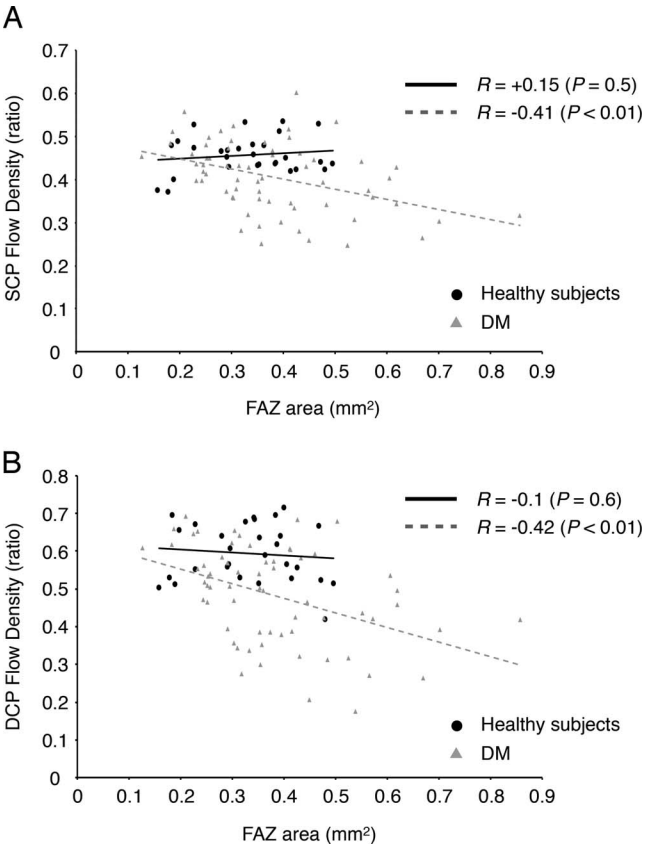

Figure 6. Correlation between the FAZ area and retinal capillary FD. The relationship between the FAZ area and the retinal capillary FD of the (**A**) SCP and (**B**) DCP were examined by Pearson's correlation coefficient analysis in healthy eyes (*black circle*: $n = 29$) and diabetic eyes (*gray triangle*: $n = 64$).

Furthermore, we examined the correlation between FD and FAZ size. In normal subjects, there was no significant correlation between FAZ and FD. This could be due to the large variability of the FAZ in normal individuals.[28] Conversely, significant correlation was found between the magnitude of FAZ and FD in DM patients. This suggests that DM could affect FAZ-forming capillaries equally as with the other macular capillaries at a 3 × 3 mm foveal area. Furthermore, our data indicate that it may be possible to predict retinal FD by measuring FAZ in DR. However, taking into consideration the fact that this is a cross-sectional study, it is necessary to conduct a follow-up study to confirm this finding. In addition, further studies should include a wider imaging area.

Previous studies have reported that the vascular disorder of DR is of a nonuniform distribution.[29] Pathologic examination has also showed that vascular abnormalities occur more frequently in the temporal retina than in the nasal retina.[18] Furthermore, a recent study using ultrawide field imaging reported that vascular lesions occurred more frequently in the temporal fields compared with the nasal fields.[19] The current study using en face 3 × 3 mm as well as 6 × 6 mm OCTA images did not show any significant difference with regard to FD among the four areas. A possible explanation is that findings such as microaneurysms used for ETDRS grade and the FD in OCTA do not necessarily coincide as spatially occurring sites. Moreover, given that it has been reported that some of the

earliest clinical changes in DR occur in the midperipheral fundus, even in the study using FA,[30] this 6 × 6 mm range of OCTA may be too small to detect the nonuniform distribution. However, the spatial bias ratio of the NPDR group was significantly higher than one of the NDR groups. Furthermore, the minimum values of FD were not equally distributed in the DCP of NPDR. Interestingly, in both the 6 × 6 mm and 3 × 3 mm OCTA analyses, the minimum value of FD was significantly higher in the temporal area. These data suggest that diabetic capillary dropout occurs in any area of the macula. Although the appearance could be random in individual cases, it is likely to occur in the temporal area. A bias of vascular disorders occurring in the deep layer was observed in this study. Past reports[17–19] have not addressed this preference of vascular disorders occurring in particular layers, and its clinical significance may be important in the future. Further investigation concerning peripheral capillaries with OCTAs is warranted.

Importantly, this spatial bias could only be detected in the DCP. The hierarchical bias of FD significantly shifted to a DCP dominance with progression of DR, a finding consistent with several papers on the deep plexus dominance of DR-related vascular disorders.[13] However, because DCP images may include projection artifacts in this study, it is also necessary to consider using a novel method to exclude such artifacts in the future for more reliable interpretation of data.[31]

This study used area-divided OCTA quantification to show that DR-associated capillary dropout can occur locally with spatial bias. However, macular capillary dropout occurs in any of the four directions. Furthermore, vascular disorders occur regardless of the anatomic specificity of FAZ. However, despite the equal exposure of the vascular endothelium to hyperglycemic conditions, the occurrence of spatial bias of capillary dropout remains unpredictable in individual cases. The mechanism underlying DR capillary dropout is due to pericyte loss and endothelial cell injury.[32] In diabetic animal models, vascular endothelial cell death occurs locally in any region[33,34] and the vascular disorder may originate locally due to involvement of leukocyte adhesion.[3] Furthermore, our previous studies with in vivo molecular imaging have reported that DR-related molecules are upregulated locally in any region.[35] Although spatial patterns of circulatory dynamics are also possible hypotheses,[36] fundamental experiments are warranted to determine the factors influencing the location of capillary dropout in each patient. It is also important to examine the clinical significance of such spatial patterns.

This study has several limitations inherent in any study of a limited sample size. Another limitation is that patients with macular edema or vitreous hemorrhage were excluded in spite of the fact that these patients represent the pathogenesis of DR. An additional limitation is the small field of view and the impact of image artifacts on FD quantification.[37] Therefore, a wider field of view using OCTA is required because DR showed vascular abnormalities in the peripheral lesions.[19,38] It is important to determine the clinical relevance of the spatial pattern of capillary dropout in DR.

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