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Samantha Fradkin

Deepthi Bannai

Paulo Lizano

Adriann Lai
Rowan University

Christen Crosta

See next page for additional authors

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Deep retinal layer microvasculature alterations in schizophrenia

Samantha I. Fradkin [a,b,c], Deepthi Bannai [d], Paulo Lizano [d,e], Adriann Lai [f], Christen Crosta [g], Judy L. Thompson [b,h], Steven M. Silverstein [b,c,h,i,*]

[a] Department of Psychology, University of Rochester, Rochester, NY, USA
[b] Department of Psychiatry, University of Rochester Medical Center, Rochester, NY, USA
[c] Center for Visual Science, University of Rochester, NY, USA
[d] Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston, MA, USA
[e] Department of Psychiatry, Harvard Medical School, Boston, MA, USA
[f] Department of Psychology, Rowan University, Glassboro, NJ, USA
[g] Department of Cell Biology and Neuroscience, Rutgers, The State University of New Jersey, Piscataway, NJ, USA
[h] Department of Neuroscience, University of Rochester Medical Center, Rochester, NY, USA
[i] Department of Ophthalmology, University of Rochester Medical Center, Rochester, NY, USA

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ABSTRACT

A subset of individuals with schizophrenia (SZ) are thought to have a microvascular component to their illness with studies demonstrating alterations in retinal superficial, deep, and choroidal microvasculature networks. However, the direction and location of these alterations have differed across studies. In a recent study, we reported that individuals with SZ demonstrated lower superficial layer perfusion density than a healthy control (HC) group. The current study investigated characteristics of the deep vascular layer in SZ. We included 28 individuals with a diagnosis of SZ or schizoaffective disorder, and 37 HCs. Optical coherence tomography angiography (OCTA) data was collected to measure deep retinal layer perfusion density, skeletonized vessel density, vessel diameter index, and fractal dimension. We conducted between-group comparisons to examine differences in these OCTA variables between SZ and HC groups. A trend analysis was conducted to determine if differences reflected a linear trend according to age and illness length, and Spearman correlations were conducted to determine associations between deep and superficial layer density. Individuals with SZ demonstrated significantly lower bilateral perfusion density and vessel diameter index, as well as lower left eye skeletonized vessel density and fractal dimension. There was a significant linear trend in the data indicating that individuals with chronic SZ demonstrated the lowest OCTA values, followed by individuals within two years of their first episode of psychosis who did not differ from older controls, followed by younger controls, who demonstrated the highest values in at least one eye. Lower density values in the deep retinal layer were also significantly associated with lower density values in the superficial layer. Overall, results suggest that microvascular alterations are present in multiple retinal layers in SZ and that they may be useful visual system biomarkers of neurovascular changes in the disorder.

Deep retinal layer microvasculature alterations in schizophrenia

Schizophrenia (SZ) is a complex psychiatric disorder that leads to shortened life expectancies, by about 15 to 20 years, compared to the general population, and cardiovascular disease has been found to be a major contributing factor (Correll et al., 2017; Hennekens et al., 2005; Laursen et al., 2014; Sweeting et al., 2013). A variety of secondary factors, including side effects of antipsychotic drugs (De Hert et al., 2012; Newcomer, 2007), lifestyle factors (De Hert et al., 2012), and healthcare disparities (Lawrence & Kisely, 2010; Mitchell and Dinan, 2010) may increase risk for cardiovascular disease in this population. However, researchers have proposed that SZ may also be, fundamentally, a microvascular disorder (Green et al., 2022; Moises et al., 2015), whereby alterations in the vascular system may lead to changes in neural health and brain structure and function (Hanson and Gottesman, 2005; Pong et al., 2020). This is supported by evidence suggesting increased permeability of the blood brain barrier and alterations in cerebral blood flow in SZ (Bishop et al., 2022; Lizano, Pong, et al., 2023;

* Correspondence to: Department of Psychology, University of Rochester, P.O. Box 270266, Rochester, NY 14627-0266, USA.
E-mail address: sfradkin@ur.rochester.edu (S.M. Silverstein).

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Ouellette and Lacoste, 2021; Puvogel et al., 2022). Genes associated with microvascular processes are also overrepresented among SZ-associated genes (Moises et al., 2015; Pillinger et al., 2023).

Examination of the retina, often referred to "a window into the brain," has been proposed as an alternative, non-invasive method to measure changes in, and enhance our understanding of, the microvascular system, brain, and overall central nervous system (CNS) structure and function (Green et al., 2022; London et al., 2013; Wagner et al., 2020). The retinal and cerebral microvasculature are homologous in a variety of anatomical and functional properties, including similar blood-retinal and blood-brain barriers, cell types, and vascular regulatory processes (Cabrera DeBuc et al., 2017; Patton et al., 2005). Further, alterations in retinal microvasculature have been found in a variety of cerebrovascular, neural, and psychiatric diseases (Doubal et al., 2009; Hilal et al., 2014; Jiang et al., 2021; Kennedy et al., 2023). Therefore, retinal microvasculature markers have been proposed as indicators of cerebral microvasculature pathology (Patton et al., 2005).

Retinal imaging is a rapid, non-invasive and inexpensive method, relative to typical brain imaging techniques (e.g., positron emission tomography and arterial spin labeling), to assess for changes in CNS structure and function (Green et al., 2022; Patton et al., 2005; Spaide et al., 2018). Initial studies of the retinal microvasculature in SZ used fundus photography to study differences in the width and network complexity of retinal venules and arterioles. These studies demonstrated that individuals with SZ are characterized by wider retinal venules (Appaji et al., 2020; Hosák et al., 2020; Liu et al., 2020; Meier et al., 2015; Meier et al., 2013). Findings focused on retinal arterioles in SZ have been mixed, as one study found narrower arterioles in SZ (Appaji et al., 2020) and other studies found wider arterioles when compared to controls (Hosák et al., 2020; Liu et al., 2020). Studies have also reported greater fractal dimension, or branching network complexity (Appaji, Nagendra, Chako, Padmanabha, Hiremath, et al., 2019b), alterations in vascular trajectory (Appaji, Nagendra, Chako, Padmanabha, Jacob, et al., 2019a), and greater retinal vascular tortuosity (Appaji, Nagendra, Chako, Padmanabha, Jacob, et al., 2019b) in SZ. Retinal microvasculature anomalies may also serve as a proxy marker of genetic risk, as unaffected twins and relatives of individuals with SZ have been shown to have widened retinal venules (Hosák et al., 2020; Meier et al., 2015) and arterioles (Hosák et al., 2020) with diameters between those of SZ and control groups.

Optical coherence tomography angiography (OCTA) is a newer (2014) and faster retinal vascular imaging technique that produces images of the retinal microvasculature and allows for the measurement of blood flow in all retinal and choroidal microvasculature layers with high resolution (Spaide et al., 2018; see Fig. 1 for display of retinal microvascular and neural layers). Using OCTA, studies have reported superficial, deep, and choroidal microvasculature density alterations in SZ and these have been observed in both macular and peripapillary regions. However, the direction and location of these alterations have differed across studies. Bannai et al. (2022) studied the macula and found that individuals with SZ demonstrated greater right eye choriocapillaris perfusion density as well as superficial and choriocapillaris vessel length compared to controls, with the greatest differences noted within early-course SZ participants. On the other hand, lower superficial layer perfusion density and vessel length have been observed in the macula of individuals with SZ (Silverstein et al., 2021). Findings from Koman-Wierdak et al. (2021) suggest that lower perfusion density is also present within the radial peripapillary capillary and macular deep vascular complex in people with SZ. When assessing specific quadrants, Budakoglu et al. (2021) found lower peripapillary vascular density within the temporal quadrant and Bannai et al. (2022) observed greater choriocapillaris network complexity in the superficial and deep layers. Taken together, the evidence suggests that there are changes in retinal microvascular network properties in SZ, with mixed findings regarding the direction (increased vs decreased) and location (macula vs peripapillary) of the changes. As discussed below and in the Discussion, these differences may reflect the vascular layer being measured, the length of illness, and the medical condition of subjects.

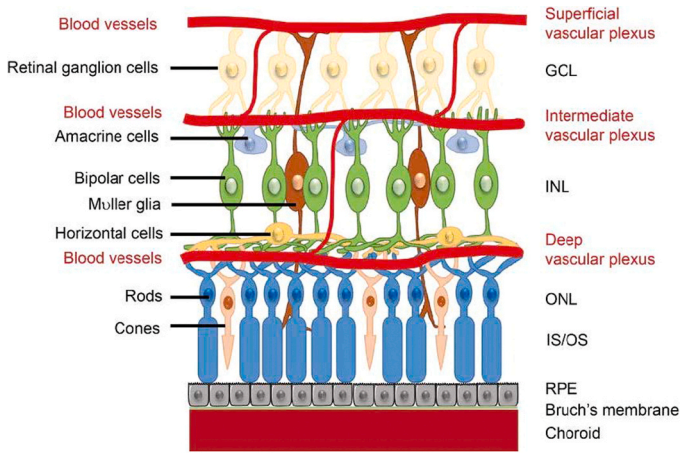

Fig. 1. Retinal neural layers and microvasculature plexuses. Note. Retinal microvasculature plexuses displayed with associated neural layers. GCL = ganglion cell layer; INL = inner nuclear layer; ONL = outer nuclear layer; IS/OS = inner segments/outer segments; RPE = retinal pigment epithelium. From "Targeting Neurovascular Interaction in Retinal Disorders," by Z. Fu, Y. Sun, B. Cakir, Y. Tomita, S. Huang, Z. Wang, C-H. Liu, S. S. Cho, W. Britton, T. Kern, D. A. Antonetti, A. Hellström, and L. E. H. Smith, 2020, *International Journal of Molecular Sciences*, 21(4) (https://doi.org/10.3390/ijms21041503). CC BY 4.0.

Evidence for changes in retinal vascular properties over the course of a disease comes from findings in Alzheimer's dementia and diabetic retinopathy. Greater vessel density has been observed in individuals with pre-clinical Alzheimer's disease (van de Kreeke et al., 2020), whereas reduced vessel density is generally found in the later stages (Pellegrini et al., 2020; Song et al., 2021). As van de Kreeke et al. (2020) notes, this could be due to inflammation, hypoxia, and increased blood flow early in the illness, with subsequent vessel atrophy. Fragiotta et al. (2023) also found an initial increase in perfusion density in individuals with mild non-proliferative diabetic retinopathy within the superficial and deep capillary plexuses one to two years following the initial assessment. At 3- and 4-year follow-up sessions, however, participants demonstrated a subsequent reduction in superficial perfusion density (Fragiotta et al., 2023). Given that studies have found both denser and thinner microvasculature networks in SZ, findings may reflect a similar disease-related trajectory. It is also possible that lower perfusion density in one layer may be compensated for by increased perfusion density in another layer, at least early in the course of illness (Lin et al., 2019). Therefore, the primary aim of the current study was to compare measures of deep retinal layer perfusion density, skeletonized vessel density, vessel width, and fractal dimension between SZ and control groups. This study performed additional analyses on data from previously published studies that reported lower superficial layer density (Silverstein et al., 2021) and retinal neural layer alterations in SZ (Lai et al., 2020). We hypothesized that individuals with SZ would demonstrate lower deep layer density values compared to healthy controls (HCs) and that these alterations would be associated with lower density values in the superficial layer. We also predicted that the SZ group would demonstrate lower vessel diameter index and fractal dimension, or network complexity, values when compared to HCs.

Method

Participants

Subjects included 28 individuals with a diagnosis of SZ or schizoaffective disorder (collectively referred to as SZ in the rest of the paper) and 37 HCs. The SZ group included 12 individuals within 2 years of

experiencing their first episode of psychosis and 16 later episode participants, and the control group included 19 participants age-matched to first episode participants and 18 participants age-matched to later episode participants. As described in Lai et al. (2020), participants within the SZ group were recruited from outpatient clinics or partial hospital programs at Rutgers University Behavioral Health Care. Participants within the healthy control group were recruited from the community via posted flyers and online advertisements. Participants were included if they were between 18 and 65 years old, could understand English, and had normal or corrected-to-normal visual acuity. Exclusion criteria included: (1) eye injury or disease (e.g., cataracts, macular degeneration, diabetic retinopathy, glaucoma); (2) neurological, intellectual, or developmental disorders; (3) psychiatric diagnoses of bipolar disorder or major depressive disorder (including past and current depressive episodes); (4) a history of head injury with loss of consciousness greater than 10 min; (5) electroconvulsive therapy in the past 8 weeks; (6) amblyopia (lazy eye) or squint; and (7) self-reported or chart diagnosis of diabetes or high blood pressure (both of which are cardiovascular risk factors that occur at elevated rates in SZ [Bresee et al., 2010; Vancampfort et al., 2016] and can independently affect the retinal microvasculature [Choi et al., 2017; Sahin et al., 2015]) (Lai et al., 2020; Silverstein et al., 2021). The study was approved by the Rutgers Biomedical and Health Sciences Institutional Review Board and written informed consent was obtained from all subjects.

Optical coherence tomography angiography (OCTA)

OCTA scans were completed using a Cirrus 5000 high definition, spectral domain device that collected data at a 2-millimeter depth and 5 micrometers resolution. OCTA focused on deep retinal layer 3×3 mm^2 scans of the foveal avascular zone, fovea, and area surrounding the fovea for both right and left eyes. Signal strength of the OCTA scans was at least 8 out of 10 for all subjects and most scans demonstrated a signal strength of at least 9 out of 10. The manufacturer of the OCT machine, Zeiss, recommends that within a clinical context, OCTA scans should have a signal strength of at least 6 out of 10, although evidence suggests that signal strength below 9 out of 10 may influence OCTA values (Lim et al., 2018; Zeiss, 2008).

The processing pipeline for the OCTA images utilized deep plexus angiography images that had larger superficial vessels removed, which was performed using the device manufacturer's software, as well as an image trace of the foveal avascular zone (FAZ), also acquired from the device. Both images were used within a custom semi-automated algorithm that processed and extracted the following morphological vascular measures: perfusion density, skeletonized vessel density, vessel diameter index, and fractal dimension (all of which are defined below; Bannai et al., 2022).

Image analysis followed several steps. First, the raw deep plexus angiography image was processed through a contrast-limited adaptive histogram equalization (CLAHE) filter to enhance contrast. Then, the background intensity, acquired from morphologically opening the image using a disk window of 12 pixels, was subtracted from the CLAHE-filtered image. The average FAZ pixel intensity was then subtracted, resulting in a globally-thresholded image that contained a true-black FAZ. From there the image was binarized and run through a Hessian-based Frangi vesselness filter (Kroon, 2024), resulting in two separate images. For one image, a maximum sigma of the Gaussian kernel for the Hessian filter was 2.5 and the image was then binarized using the MATLAB 'imbinarize' function. The second image, which was globally-thresholded, was similarly binarized utilizing a threshold of 0.28. These two images were combined where pixels overlapped in both the binarized-Hessian-filtered and binarized-only images. Vessel elements with connected components smaller than 20 pixels were removed from the binarized image using 'bwareaopen.' Skeletonizing the images was performed using the MATLAB function 'bwmorph' and the 'skel' operation. This allowed for the pixel-wise removal of vessels until reaching a thickness of 1 pixel without disturbing the continuity of the vessel structure. Retinal vascular measures were then extracted from the final binarized and skeletonized images.

Microvascular measures were extracted, using methodology by Kim et al. (2016) from this final binarized image. Perfusion density was quantified as the ratio between the area encompassed by the vessels (total number of white pixels) and the total area of the image (total number of pixels). Skeletonized vessel density (i.e., vessel length) was computed as the total amount of white pixels divided by the total area of the skeletonized image. Vessel diameter index (i.e., vessel width) was calculated as perfusion density divided by skeletonized vessel density, or vessel area divided by vessel length. Finally, the box-counting method (Chen et al., 1993) was performed on the skeletonized image to calculate fractal dimension (i.e., network complexity).

Data analytic approach

Statistical analyses were completed using IBM SPSS Statistics, Version 28.0.0.0. Between-group analyses were conducted to determine differences in deep retinal layer perfusion density, skeletonized vessel density, vessel diameter index, and fractal dimension between the SZ and HC groups. Group differences were analyzed with t-tests when all assumptions were met. Non-parametric tests, including the Mann-Whitney U test, were used when the sample was not normally distributed and the Welch's F test was used when the assumption of homogeneity of variance was violated. We also conducted a trend analysis (Rosenthal and Rosnow, 1985) to test whether degree of retinal abnormality was a function of both age and illness length. Specifically, we tested whether the pattern of the four group means (non-first episode SZ, first-episode psychosis, older controls, and younger controls) fit a polynomial (linear) trend with contrast weights of -2, 0, 0, and 2, respectively. To examine the association between deep retinal and superficial layer microvasculature perfusion and skeletonized vessel density, we conducted bivariate Spearman correlations.

Results

Demographic characteristics

Demographic information is displayed in Table 1. There were no significant SZ-HC differences in age, maternal education or paternal education. As is typical, due to illness onset interfering with educational attainment (Meehl, 1971), individuals with SZ demonstrated fewer years of education than the control group ($p < 0.001$).

Table 1
Demographics.

Variable	SZ	HC
N	28	37
Sex		
Female	4	13
Male	24	24
Age (M ± SD)	32.21 ± 11.27	32.2 ± 12.63
Race		
White	6	24
Black or African American	16	3
Asian	3	6
Native Hawaiian or Other Pacific Islander	0	1
Mixed Race	1	3
Ethnicity		
Hispanic	5	5
Non-Hispanic	23	32
Participant education (M ± SD)	15.34 ± 2.57	17.16 ± 2.02
Maternal education (M ± SD)	14.90 ± 2.64	15.22 ± 3.00
Paternal education (M ± SD)	15.16 ± 2.48	15.35 ± 3.31

Note. SZ = Schizophrenia/schizoaffective; HC = Healthy control. Two participants within the schizophrenia group did not report race.

Between group differences

Compared to HCs, individuals with SZ demonstrated lower values for all four deep retinal layer OCTA variables in at least one eye (see Table 2 for mean, standard deviation, and median values). The SZ group showed lower right eye, $U = 315.00$, $z = -2.04$, exact $p = .041$, $\eta^2 = 0.07$, and left eye perfusion density compared to controls, *Welch's F* (1, 38.97) $= 7.85$, $p = .01$, $\eta^2 = 0.13$. Individuals with SZ also demonstrated lower vessel diameter index (i.e., vessel width) for right eye, $t(59) = 3.24$, $p = .002$, $d = 0.84$ and left eye data compared to controls, $t(60) = 2.61$, $p = .01$, $d = 0.67$. For the left eye only, individuals with SZ demonstrated lower skeletonized vessel density (i.e., total vessel length), *Welch's F* (1, 43.69) $= 6.60$, $p = .01$, $\eta^2 = 0.11$ and lower fractal dimension (i.e., vessel network complexity), *Welch's F* (1, 39.93) $= 7.14$, $p = .01$, $\eta^2 = 0.28$. For the right eye, the SZ group demonstrated marginally lower skeletonized vessel density than the control group ($p = 0.10$) and non-significantly lower fractal dimension ($p = 0.12$). See Fig. 2 for graphical representation of significant statistical differences of deep layer microvasculature variables between SZ and HC groups.

Contrast analyses

Contrasts were calculated to examine whether deep retinal layer microvasculature density, vessel diameter index, and fractal dimension decreased as a function of age and illness length. The hypothesized model predicted that younger controls would demonstrate the greatest OCTA values, older individuals with chronic SZ would demonstrate the lowest OCTA values, and individuals with first episode psychosis and older controls would demonstrate OCTA values in between these two groups. Planned contrasts revealed that right and left eye perfusion density ($ps < 0.05$, $\eta^2_p > 0.07$) and vessel diameter index ($ps < 0.01$, $\eta^2_p > 0.13$), as well as left eye skeletonized vessel density ($p < 0.001$, $\eta^2_p = 0.18$) and fractal dimension ($p < 0.001$, $\eta^2_p = 0.19$), decreased significantly as a function of age and length of illness. See Table 3 for results for all OCTA variables tested and Fig. 3 for graphical representation of the linear trend for left eye perfusion density. Right eye skeletonized vessel density demonstrated a similar pattern across the four age groups at a trend level ($p = 0.09$, $\eta^2_p = 0.05$) and right eye fractal dimension did not show a significant linear trend ($p = 0.14$, $\eta^2_p = 0.04$), although both of these effect sizes were within the small-medium range.

Relationships between deep retinal and superficial layers

Spearman correlations were conducted to determine whether deep retinal layer perfusion and skeletonized vessel density were associated with the superficial layer values reported in Silverstein et al. (2021). Results indicated that for the SZ group, lower perfusion density in the deep retinal layer was associated with lower perfusion density in the superficial layer for both the right ($r_s = 0.73$, $p < 0.001$) and left eye ($r_s = 0.82$, $p < 0.001$). Similarly, the SZ group demonstrated associations between lower skeletonized vessel density in the superficial and deep retinal layers for right ($r_s = 0.63$, $p < 0.001$) and left eye ($r_s = 0.75$, $p < 0.001$) data (see Fig. 4 for scatterplots that illustrate these relationships). These results suggest that lower perfusion and skeletonized vessel density are present throughout multiple retinal layers within individuals with SZ. The control group also demonstrated these associations for right eye perfusion ($r_s = 0.66$, $p < 0.001$) and skeletonized vessel density ($r_s = 0.70$, $p < 0.001$), but these associations were not observed for left eye perfusion ($p = 0.18$) and skeletonized ($p = 0.51$) vessel density. The earlier study did not acquire data on vessel width or fractal dimension and so no comparisons were possible for these variables.

Table 2
Description Statistics for Study Metrics.

Retinal layer variable	SZ		HC	
	M (SD)	*Mdn*	*M (SD)*	*Mdn*
Right eye				
Perfusion density	0.27 (0.04)	0.28	0.29 (0.05)	0.30
Skeletonized vessel density	0.06 (0.01)	0.06	0.06 (0.01)	0.07
Vessel diameter index	4.46 (0.10)	4.46	4.53 (0.08)	4.45
Fractal dimension	1.65 (0.03)	1.67	1.67 (0.03)	1.67
Left eye				
Perfusion density	0.27 (0.04)	0.26	0.30 (0.03)	0.30
Skeletonized vessel density	0.06 (0.01)	0.06	0.07 (0.01)	0.07
Vessel diameter index	4.46 (0.10)	4.45	4.52 (0.09)	4.53
Fractal dimension	1.65 (0.02)	1.65	1.67 (0.02)	1.67

Note. SZ = Schizophrenia/schizoaffective; HC = Healthy control; *M* = Mean; *Mdn* = Median; *SD* = standard deviation

Discussion

The present study investigated whether microvascular indices within the deep retinal layer differed between SZ and HC groups. Secondary analyses were performed on data from a previously published study that found lower perfusion density and vessel length in the superficial vascular layer in SZ (Silverstein et al., 2021). Findings from the current study indicated that individuals with SZ demonstrated lower bilateral perfusion density and vessel width as well as lower left eye vessel length and vessel network complexity in the deep vascular layer compared to HCs. In addition, microvasculature changes were characterized by a linear trend, suggesting a progressive loss of retinal microvasculature with increasing age and passage beyond the second psychotic episode. We also observed that lower deep layer perfusion density and vessel length values were significantly correlated with lower values within the superficial vascular layer. Overall, findings suggest that retinal microvasculature alterations are present in both the superficial and deep retinal layers in SZ.

The findings herein demonstrating lower deep retinal layer perfusion density and vessel length in SZ are consistent with findings from previous studies that showed lower density within the deep vascular complex (Koman-Wierdak et al., 2021), peripapillary region (Budakoglu et al., 2021; Koman-Wierdak et al., 2021), and superficial layer (Silverstein et al., 2021). However, these findings differed from the greater choriocapillaris perfusion density and superficial and choriocapillaris vessel length observed by Bannai et al. (2022). Although the reason for the differences in these results is unclear, it is possible that vascular structure in the choroid is characterized by a different trajectory of change than is vasculature in the neural layers of the retina. Findings by Li et al. (2022) support that the choroid may reflect a different trajectory of change, as they found lower choroid vascular density in males with psychosis and lower choroid vascular volume and choroid thickness in females with psychosis when compared to same-sex controls. The researchers did not observe these differences in choroid microvasculature when comparing all individuals with psychosis and controls. Additionally, as Bannai et al. (2022) reported, these differences could be due to differences in exclusion criteria, they did not exclude individuals with diabetes and hypertension. It is important to note that Bannai et al. (2022) observed the greatest density and network complexity values among early-course SZ participants. Similarly, the current study found that deep retinal layer perfusion density (bilateral), vessel width (bilateral), vessel length (left eye) and network complexity (left eye) decreased as a function of age and illness duration. These results suggest that microvascular networks are increasingly damaged as the disorder progresses. As van de Kreeke et al. (2020) suggested occurs in Alzheimer's disorder, it is possible that in SZ, a combination of inflammatory processes (Dunleavy et al., 2022; Lizano, Kiely, et al., 2023) and hypoxia (Moises et al., 2015) could lead to initial increase in blood flow during and/or following a first episode of psychosis, eventually followed by microvascular damage and reductions in blood flow.

The finding of a smaller vessel diameter index in SZ suggests the presence of narrower retinal vessels within the disorder. This is

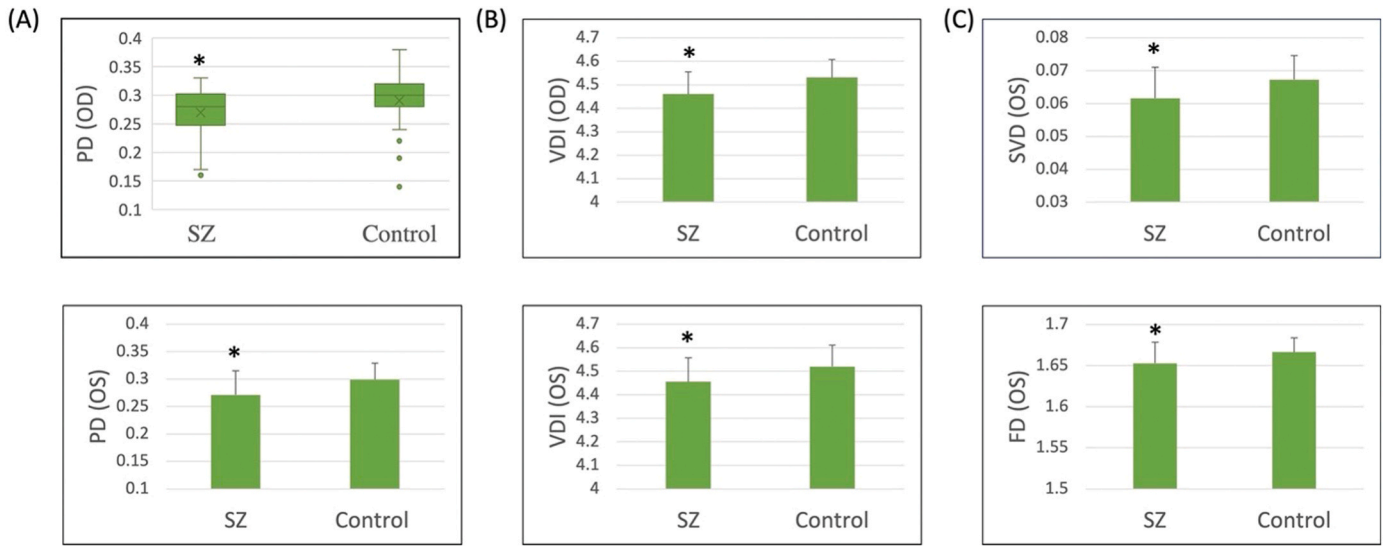

Fig. 2. Graphical representation of deep retinal layer microvasculature comparisons between schizophrenia and control groups. Box plot and bar charts for deep retinal layer microvasculature indices that demonstrated significant differences between schizophrenia and control groups. Error bars reflect 1 standard deviation. Comparison of right eye perfusion density data (A) is reflected as a box plot, as median values were compared between groups due to non-normal distribution of data. SZ = schizophrenia/schizoaffective; OD = oculus dexter (right eye); OS = oculus sinister (left eye); PD = perfusion density (A); VDI = vessel diameter index (B); SVD = skeletonized vessel density (C); FD = fractal dimension (C).

Table 3
Results of planned contrast analyses of retinal measures by age and illness length.

	SS	df	MS	F	p	η_p^2
PD_OD	0.01	1, 57	0.01	4.22	0.05 *	0.07
PD_OS	0.02	1, 58	0.02	15.92	< .001 * *	0.22
SVD_OD	0.00	1, 57	0.00	3.11	0.09 ~	0.05
SVD_OS	0.00	1, 58	0.00	12.87	< .001 * *	0.18
VDI_OD	0.06	1, 57	0.06	8.27	0.01 *	0.13
VDI_OS	0.08	1, 58	0.08	9.44	0.003 *	0.14
FD_OD	0.00	1, 57	0.00	2.25	0.14	0.04
FD_OS	0.01	1, 58	0.01	13.80	< .001 * *	0.19

Note. SS = Sum of squares; MS = Mean square; OD = oculus dexter (right eye); OS = oculus sinister (left eye); PD = perfusion density; SD = skeletonized vessel density; VDI = vessel diameter index; FD = fractal dimension. This table displays results of planned contrast analyses that tested whether the four group means (non-first episode SZ, first-episode psychosis, older controls, and younger controls) fit a linear trend with contrast weights of − 2, 0, 0, and 2, respectively, for the four OCTA variables. Results demonstrated that the retinal vascular measures decreased significantly or at a trend level as a function of age and illness length for seven out of eight variables.
Test statistics at trend level are denoted as ~. Significant test statistics are denoted as * $p < 0.05$; * * $p < 0.001$.

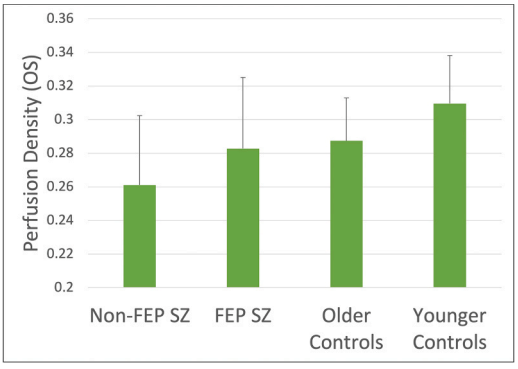

Fig. 3. Graphical representation of left eye perfusion density according to age and illness length. Note. Bar chart that shows that left eye perfusion density decreased linearly as a function of age and illness length ($p < 0.001$). FEP = first episode psychosis; SZ = schizophrenia; OS = oculus sinister (left eye).

consistent with findings from Appaji, Nagendra, Chako, Padmanabha, Hiremath et al. (2019a) who observed narrower arterioles in SZ on fundus photographs. On the other hand, this differs from findings from Bannai et al. (2022) where, using OCTA, they found no differences in vessel diameter index between individuals with SZ and controls. In addition, using fundus photography, other studies have even observed widened retinal venules in SZ (Appaji, Nagendra, Chako, Padmanabha, Hiremath, et al., 2019a; Hosák et al., 2020; Meier et al., 2015; Meier et al., 2013). However, due to the technology used, these studies did not measure capillary width within the deep vascular layer. Therefore, differences between the current findings and previous studies may reflect differences in the location and types of blood vessels measured.

This study was characterized by a number of limitations. One is the relatively small sample size. Another was the use of data from a sample with known smaller superficial layer microvasculature measurements. Therefore, replication with a larger and new sample is needed. Another limitation is that, while study criteria ruled out subjects with diabetes or hypertension, blood pressure and metabolic indicators (e.g., A1C level) were not measured. It will be useful for future studies to clarify relationships between these variables and retinal markers. The study also did not collect data on various other factors that are common in SZ and that are known to influence health of the retina, such as smoking, body mass index, and sleep apnea (Silverstein et al., 2020). Therefore, future studies that quantify these variables and include them in prediction models will allow for a determination of the extent to which the retinal vascular changes observed in SZ are independent of these additional factors.

If future studies demonstrate that retinal vascular changes are more strongly related to SZ itself than to other effects, these changes may serve as useful biomarkers of disease progression, and may even be useful in calculating risk for outcomes such as an initial psychotic episode or relapse. Longitudinal studies, in particular, are needed to examine the predictive validity of these retinal vascular changes on clinical outcomes. Even if a significant proportion of retinal vascular effects are determined to be secondary to 'comorbid' factors (which may nevertheless share genetic variance with SZ, such as cardiovascular

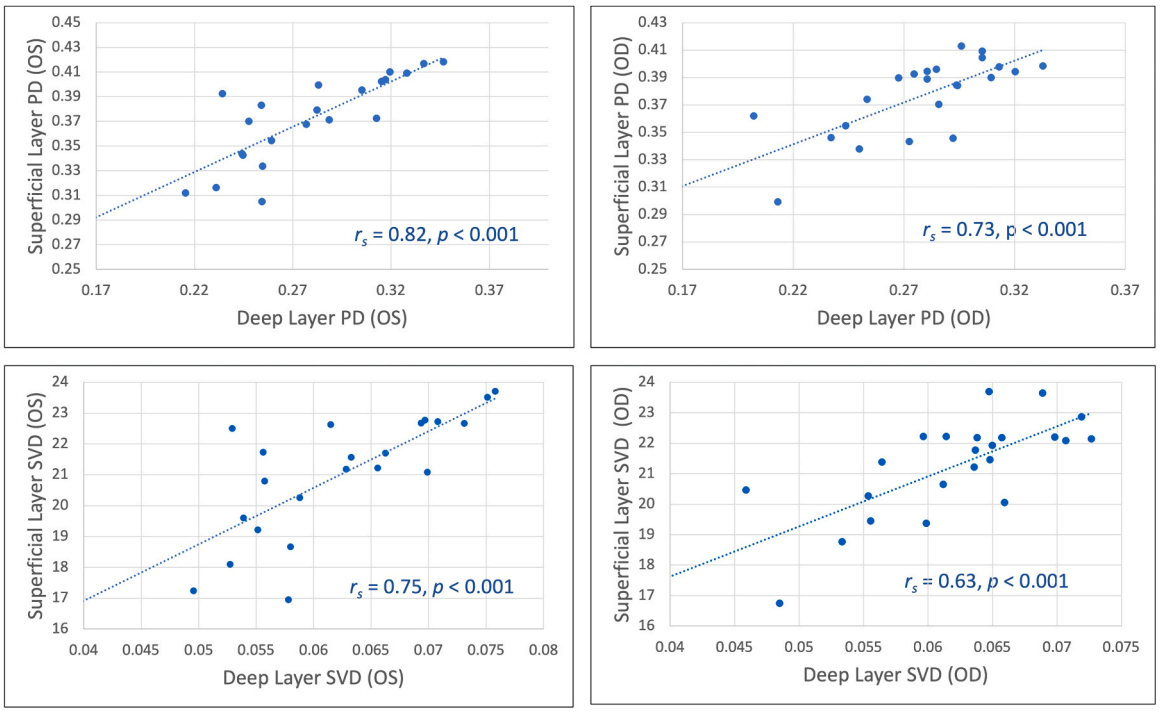

Fig. 4. Association between deep retinal and superficial layer density values. Note. Scatter plots that display significant associations between deep retinal and superficial layer perfusion and skeletonized vessel density for the schizophrenia group. OS = oculus sinister (left eye); OD = oculus dexter (right eye); PD = perfusion density; SVD = skeletonized vessel density.

(Moises et al., 2015; Pillinger et al., 2023) and metabolic (Malan-Müller et al., 2016) disease), it is still possible that OCTA may be useful in understanding overall CNS functioning in individuals with SZ. In real-world clinics, many SZ patients present with multiple medical issues such as diabetes, high blood pressure, sleep disturbances, high smoking rates, and obesity. Therefore, OCTA may be useful for patient monitoring and prediction efforts, or in characterizing heterogeneity within SZ, even if findings in SZ are not diagnostically specific in that they overlap with those from other central nervous system disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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