

Retinal Microvascular Signatures and Retina–Brain Coupling Across Cerebrovascular Disease Subtypes: An OCTA and Quantitative MRI Study

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Supplementary Methods

Structural MRI Acquisition Parameters

Structural T1-weighted images were acquired using inversion-recovery T1-weighted sequences, including vendor-specific T1-FLAIR implementations. Representative acquisition parameters included: a repetition time (TR) ranging from 2000 to 3100 ms, an echo time (TE) of approximately 20–24 ms, and an inversion time (TI) ranging from 850 to 985 ms. The flip angle varied between 90° and 111°, contingent upon vendor-specific optimizations. The geometric parameters typically consisted of 21–22 axial slices with a slice thickness of 5–6 mm and variable inter-slice gaps (0.5–1.5 mm). The in-plane resolution ranged from $0.47 \times 0.47 \text{ mm}^2$ to $0.60 \times 0.60 \text{ mm}^2$.

Structural T2-weighted FLAIR images were acquired using standard turbo spin-echo or fast spin-echo sequences. The representative acquisition parameters were defined as follows: TR = 6000–8500 ms, TE = 80–150 ms, and TI = 2000–2500 ms, with flip angles ranging from 90° to 150°. The spatial geometric parameters for the T2-FLAIR sequences—including the X-Y in-plane resolution, the number of axial slices, the slice thickness, and the inter-slice gap—were strictly identical to those utilized for the corresponding T1-weighted acquisitions.

The aggregation strategy of regions of interest (ROIs)

The aggregation strategy was as follows: (1) Lobar cortical ROIs: Cortical parcellations were grouped into major lobes for each hemisphere, including the frontal, parietal, temporal, occipital, cingulate, and insular cortices. (2) Subcortical ROIs: The basal ganglia ROI was defined as the sum of the caudate, putamen, pallidum, and nucleus accumbens. The hippocampus-amygdala complex (referred to as the hippocampus ROI) combined the hippocampus and amygdala. The thalamus was analyzed as a distinct structure. (3) Infratentorial structures: The cerebellum ROI combined both cerebellar cortex and white matter, and the brainstem was assessed as a whole.

Supplementary Table 1: Pairwise comparisons of OCT/OCTA and MRI-derived brain imaging metrics among HC, CAS, RSSI, and CSVD groups

Variables	HC	CAS	RSSI	CSVD	HC vs CAS	HC vs RSSI	HC vs CSVD	CAS vs RSSI	RSSI vs CSVD
OCT/OCTA metrics									
RNFL Thickness, μm	38.64 $\pm$ 7.84	39.72 $\pm$ 8.31	37.87 $\pm$ 3.81	38.2 $\pm$ 5.45	0.835	0.094	0.270	0.034	0.600
GCIPL Thickness, μm	65.53 $\pm$ 7.14	62.97 $\pm$ 7.78	66.35 $\pm$ 7.22	64.74 $\pm$ 6.51	<0.001	0.184	0.390	<0.001	0.027
SVC VLD, mm^{-1}	20.2 $\pm$ 2.38	18.03 $\pm$ 2.84	20.49 $\pm$ 2.56	19.7 $\pm$ 2.36	<0.001	0.053	0.113	<0.001	0.002
DVC VLD, mm^{-1}	27.17 $\pm$ 1.96	25.52 $\pm$ 3	27.22 $\pm$ 2.01	26.47 $\pm$ 2.86	<0.001	0.290	0.043	<0.001	0.004
CC VLD, mm^{-1}	32.35 $\pm$ 2.75	30.36 $\pm$ 3.31	32.58 $\pm$ 2.67	31.91 $\pm$ 3.34	<0.001	0.019	0.176	<0.001	0.004
MRI metrics									
GM/ICV, %	35.59 $\pm$ 0.73	35.03 $\pm$ 0.95	36.06 $\pm$ 0.8	35.13 $\pm$ 0.74	<0.001	<0.001	0.066	<0.001	<0.001
WM/ICV, %	31.34 $\pm$ 0.94	30.57 $\pm$ 1.03	31.37 $\pm$ 0.78	31.33 $\pm$ 0.86	<0.001	0.744	0.160	<0.001	0.467
Total WMH/ICV, %	0.38 $\pm$ 0.08	0.99 $\pm$ 0.44	1.06 $\pm$ 0.47	0.76 $\pm$ 0.27	<0.001	<0.001	<0.001	0.068	<0.001
PWMH/ICV, %	0.36 $\pm$ 0.08	0.85 $\pm$ 0.37	0.9 $\pm$ 0.38	0.68 $\pm$ 0.24	<0.001	<0.001	<0.001	0.071	<0.001
DWMH/ICV, %	0.02 $\pm$ 0.01	0.14 $\pm$ 0.13	0.17 $\pm$ 0.13	0.08 $\pm$ 0.07	<0.001	<0.001	<0.001	0.213	0.002
Cerebellum/ICV, %	8.85 $\pm$ 0.61	8.88 $\pm$ 0.66	8.82 $\pm$ 0.63	8.8 $\pm$ 0.53	0.265	0.879	0.845	0.360	0.927
Thalamus/ICV, %	0.91 $\pm$ 0.06	0.89 $\pm$ 0.08	0.96 $\pm$ 0.08	0.88 $\pm$ 0.06	0.127	<0.001	0.403	<0.001	<0.001
Brainstem/ICV, %	1.62 $\pm$ 0.1	1.56 $\pm$ 0.11	1.62 $\pm$ 0.09	1.58 $\pm$ 0.11	0.002	0.367	0.344	0.001	0.194
Basal ganglia/ICV, %	1.45 $\pm$ 0.1	1.33 $\pm$ 0.13	1.5 $\pm$ 0.11	1.41 $\pm$ 0.11	<0.001	0.001	0.739	<0.001	0.007
Hippocampus/ICV, %	0.74 $\pm$ 0.05	0.71 $\pm$ 0.06	0.74 $\pm$ 0.05	0.7 $\pm$ 0.05	<0.001	0.571	<0.001	0.013	0.043
Temporal lobe/ICV, %	8.22 $\pm$ 0.26	8.19 $\pm$ 0.29	8.36 $\pm$ 0.32	8.08 $\pm$ 0.23	0.684	0.001	0.042	<0.001	0.001
Frontal lobe/ICV, %	12.17 $\pm$ 0.41	12.02 $\pm$ 0.51	12.42 $\pm$ 0.48	12.03 $\pm$ 0.47	0.010	0.001	0.698	<0.001	0.061
Parietal lobe/ICV, %	8.41 $\pm$ 0.31	8.27 $\pm$ 0.36	8.52 $\pm$ 0.32	8.35 $\pm$ 0.29	<0.001	0.086	0.742	<0.001	0.127
Occipital lobe/ICV, %	3.88 $\pm$ 0.23	3.72 $\pm$ 0.25	3.82 $\pm$ 0.25	3.81 $\pm$ 0.24	<0.001	0.126	0.182	0.033	0.545
Cingulate/ICV, %	1.85 $\pm$ 0.06	1.79 $\pm$ 0.07	1.88 $\pm$ 0.09	1.81 $\pm$ 0.06	<0.001	0.001	0.011	<0.001	<0.001
Insula lobe/ICV, %	1.06 $\pm$ 0.04	1.05 $\pm$ 0.05	1.07 $\pm$ 0.05	1.05 $\pm$ 0.05	0.739	0.007	0.346	0.005	0.101

Values are presented as mean $\pm$ standard deviation. Pairwise comparisons were performed using generalized estimating equations (OCT/OCTA metrics) or multivariable linear models (MRI metrics) adjusted for age, sex, hypertension, diabetes, dyslipidemia, smoking, and drinking, followed by false discovery rate correction. All MRI-derived regional brain volumes were normalized to intracranial volume and are expressed as percentages.

Abbreviations: CAS, carotid artery stenosis-associated stroke; CC, choriocapillaris; CSVD, cerebral small vessel disease; DVC, deep vascular complex; DWMH, deep white matter hyperintensity; GCIPL, ganglion cell–inner plexiform layer; GM, gray matter; HC, healthy control; ICV, intracranial volume; OCT, optical coherence tomography; OCTA, optical coherence tomography angiography; PWMH, periventricular white matter hyperintensity; RNFL, retinal nerve fiber layer; RSSI, recent small subcortical infarct; SVC, superficial vascular complex; VLD, vessel length density; WM, white matter; WMH, white matter hyperintensity.

Supplementary Table 2: Group-specific associations between OCT/OCTA metrics and MRI-derived brain imaging metrics in patient groups

MRI metrics	OCT/OCTA metrics	β -RSSI	β -CAS	β -CSVD	P interaction: CAS vs RSSI	for P interaction: CSVD vs RSSI
GM/ICV	RNFL Thickness	0.148(-0.1, 0.396)	0.008(-0.055, 0.072)	-0.028(-0.23, 0.175)	0.283	0.281
	GCIPL Thickness	0.026(-0.111, 0.163)	0.089(0.018, 0.159)	0.159(-0.018, 0.337)	0.424	0.242
	SVC VLD	0.06(-0.086, 0.205)	0.238(0.164, 0.311)	0.109(-0.08, 0.297)	0.032	0.686
	DVC VLD	0.042(-0.142, 0.226)	0.174(0.105, 0.244)	0.188(0.026, 0.35)	0.184	0.240
	CC VLD	0.176(0.016, 0.335)	0.281(0.207, 0.354)	0.117(-0.033, 0.266)	0.236	0.598
WM/ICV	RNFL Thickness	-0.036(-0.3, 0.228)	0.103(0.035, 0.17)	-0.115(-0.33, 0.101)	0.318	0.652
	GCIPL Thickness	0.104(-0.042, 0.25)	0.101(0.025, 0.177)	-0.038(-0.228, 0.152)	0.970	0.246
	SVC VLD	0.139(-0.018, 0.296)	0.182(0.103, 0.262)	0.064(-0.14, 0.267)	0.625	0.566
	DVC VLD	0.183(-0.015, 0.381)	0.135(0.06, 0.21)	0.113(-0.062, 0.287)	0.654	0.599
	CC VLD	0.148(-0.022, 0.318)	0.325(0.247, 0.402)	0.163(0.005, 0.322)	0.061	0.896
Total WMH/ICV	RNFL Thickness	-0.057(-0.336, 0.223)	-0.026(-0.098, 0.046)	-0.053(-0.281, 0.176)	0.835	0.983
	GCIPL Thickness	-0.051(-0.205, 0.103)	-0.119(-0.199, -0.039)	-0.064(-0.264, 0.136)	0.446	0.922
	SVC VLD	-0.001(-0.166, 0.163)	-0.222(-0.305, -0.138)	-0.141(-0.355, 0.073)	0.019	0.309
	DVC VLD	0.051(-0.158, 0.26)	-0.142(-0.221, -0.063)	-0.082(-0.267, 0.102)	0.090	0.347
	CC VLD	-0.245(-0.429, -0.06)	-0.142(-0.226, -0.058)	-0.107(-0.279, 0.065)	0.315	0.284
PWMH/ICV	RNFL Thickness	-0.076(-0.355, 0.203)	-0.049(-0.121, 0.022)	-0.053(-0.281, 0.175)	0.854	0.901
	GCIPL Thickness	-0.081(-0.234, 0.073)	-0.138(-0.217, -0.058)	-0.046(-0.245, 0.154)	0.520	0.785
	SVC VLD	-0.055(-0.219, 0.109)	-0.242(-0.325, -0.159)	-0.153(-0.366, 0.06)	0.046	0.473
	DVC VLD	-0.006(-0.215, 0.202)	-0.161(-0.239, -0.083)	-0.103(-0.286, 0.08)	0.172	0.492
	CC VLD	-0.282(-0.465, -0.099)	-0.192(-0.275, -0.108)	-0.099(-0.269, 0.072)	0.376	0.151

DWMH/ICV	RNFL Thickness	0.02(-0.261, 0.301)	0.049(-0.023, 0.121)	-0.031(-0.261, 0.198)	0.842	0.781
	GCIPL Thickness	0.052(-0.103, 0.207)	-0.022(-0.102, 0.059)	-0.092(-0.294, 0.11)	0.410	0.268
	SVC VLD	0.151(-0.015, 0.318)	-0.081(-0.165, 0.003)	-0.055(-0.271, 0.161)	0.014	0.137
	DVC VLD	0.195(-0.016, 0.406)	-0.034(-0.113, 0.046)	0.006(-0.179, 0.192)	0.046	0.187
	CC VLD	-0.048(-0.233, 0.136)	0.061(-0.024, 0.146)	-0.09(-0.262, 0.082)	0.286	0.748

Values are standardized β coefficients with 95% confidence intervals. For each OCT/OCTA metric and MRI metric pair, generalized estimating equations were fitted with standardized OCT/OCTA metrics as dependent variables and standardized MRI metrics, disease group, and MRI metric $\times$ disease group interaction terms as independent variables. RSSI was used as the reference group. Models were adjusted for age, sex, hypertension, diabetes, dyslipidemia, smoking and drinking. The group-specific β values represent the association between each MRI-derived metric and each OCT/OCTA metric within RSSI, CAS, and CSVD groups. Interaction P values indicate whether the association slope differed between CAS and RSSI or between CSVD and RSSI. P values were adjusted using the false discovery rate method. All MRI-derived GM, WM, and WMH metrics were normalized to intracranial volume.

Abbreviations: CAS, carotid artery stenosis-associated stroke; CC, choriocapillaris; CSVD, cerebral small vessel disease; DVC, deep vascular complex; DWMH, deep white matter hyperintensity; GCIPL, ganglion cell–inner plexiform layer; GM, gray matter; ICV, intracranial volume; OCT, optical coherence tomography; OCTA, optical coherence tomography angiography; PWMH, periventricular white matter hyperintensity; RNFL, retinal nerve fiber layer; RSSI, recent small subcortical infarct; SVC, superficial vascular complex; VLD, vessel length density; WM, white matter; WMH, white matter hyperintensity.

Supplementary Table 3. Group-specific associations between SVC VLD and MRI-derived regional brain volumes in patient groups

Variables	β -RSSI	β -CAS	β -CSVD	P for interaction: CAS vs RSSI	P for interaction: CSVD vs RSSI
Cerebellum/ICV, %	0.092(-0.047, 0.232)	0.017(-0.058, 0.092)	-0.014(-0.218, 0.191)	0.348	0.401
Thalamus/ICV, %	0.146(-0.001, 0.292)	0.248(0.165, 0.331)	0.032(-0.193, 0.257)	0.230	0.406
Brainstem/ICV, %	-0.165(-0.305, -0.026)	-0.031(-0.114, 0.053)	-0.073(-0.257, 0.112)	0.091	0.419
Basal ganglia/ICV, %	0.30(0.12, 0.48)	0.12(0.034, 0.207)	0.069(-0.139, 0.278)	0.046	0.115
Hippocampus/ICV, %	-0.008(-0.18, 0.164)	0.194(0.12, 0.268)	0.202(0.03, 0.375)	0.034	0.090
Temporal lobe/ICV, %	-0.053(-0.18, 0.074)	0.118(0.037, 0.2)	0.161(-0.05, 0.373)	0.026	0.089
Frontal lobe/ICV, %	0.086(-0.065, 0.236)	0.184(0.103, 0.265)	0.007(-0.17, 0.184)	0.257	0.506
Parietal lobe/ICV, %	-0.001(-0.151, 0.149)	0.148(0.072, 0.225)	0.099(-0.104, 0.301)	0.083	0.441
Occipital lobe/ICV, %	0.109(-0.024, 0.243)	0.185(0.107, 0.263)	0.089(-0.093, 0.271)	0.338	0.859
Cingulate/ICV, %	-0.014(-0.137, 0.109)	0.177(0.083, 0.271)	0.062(-0.167, 0.292)	0.014	0.563
Insula lobe/ICV, %	0.039(-0.115, 0.193)	0.049(-0.03, 0.127)	-0.015(-0.183, 0.154)	0.912	0.643

Values are standardized β coefficients with 95% confidence intervals. For each regional brain volume, generalized estimating equations were fitted with standardized SVC VLD as the dependent variable and standardized regional brain volume, disease group, and regional brain volume $\times$ disease group interaction terms as independent variables. RSSI was used as the reference group. Models were adjusted for age, sex, hypertension, diabetes, dyslipidemia, smoking and drinking. The group-specific β values represent the association between each MRI-derived regional brain volume and superficial vascular complex VLD within RSSI, CAS, and CSVD groups. Interaction P values indicate whether the association slope differed between CAS and RSSI or between CSVD and RSSI. P values were adjusted using the false discovery rate method. All MRI-derived regional brain volumes were normalized to intracranial volume and are expressed as percentages. Abbreviations: CAS, carotid artery stenosis-associated stroke; CSVD, cerebral small vessel disease; ICV, intracranial volume; MRI, magnetic resonance imaging; OCTA, optical coherence tomography angiography; RSSI, recent small subcortical infarct; SVC, superficial vascular complex; VLD, vessel length density.