Supplementary Files

Supplementary Table 1. Clinicopathologic details of 27 subjects

Supplementary Table 2. Primary antibodies and antigen recovery protocols

Supplementary Table 3. The relative consistency of arteriosclerosis and venular collagenosis burden in each region and the whole brain

Supplementary Figure 1. Associations between myelin loss using various evaluation methods and arteriosclerosis, venular collagenosis in different brain regions

Supplementary Figure 2. The correlation of arteriosclerosis or venular collagenosis burden between each region

Supplementary Figure 3. Arteriosclerosis and venular collagenosis increased with age

Supplementary Table 1. Clinicopathologic details of 27 subjects

No.	Age (y)	Sex	History[a]	Brain weight (g)	Lacunes[b]	Postmortem delay (h)
1	73	M	3	-	6	17.0
2	89	F	3	1074	-	5.0
3	79	F	1	1120	2	13.0
4	86	F	3	1020	-	6.3
5	100	F	3	-	7	3.0
6	87	M	0	1280	0	11.0
7	55	F	1	1228	2	4.0
8	79	F	5	1008	1	11.0
9	63	M	1	1267	0	4.5
10	72	M	3	1220	8	-
11	91	F	5	999	1	3.0
12	54	M	5	-	5	5.0
13	53	M	1	1278	2	8.0
14	96	F	5	1124	0	32.0
15	62	M	1	1371	2	18.5
16	64	F	1	1071	0	10.0
17	86	M	5	1044	13	5.0
18	74	F	1	1255	1	21.0
19	78	M	1	1165	3	21.5
20	87	F	5	1008	2	6.0
21	87	M	3	1154	3	8.8
22	72	M	1	1235	0	19.3
23	78	M	3	1154	0	13.5
24	95	F	1	1066	3	3.0
25	52	F	5	1201	0	22.7
26	89	M	5	1046	0	3.9
27	87	F	4	953	0	2.5

M, male; F, female.

[a] Case history was documented as 1 = tumor, 2 = stroke, 3 = dementia, 4 = coronary heart disease, 5 = others.

[b] Observed number of lacunes was recorded.

[c] – indicates the data is not available.

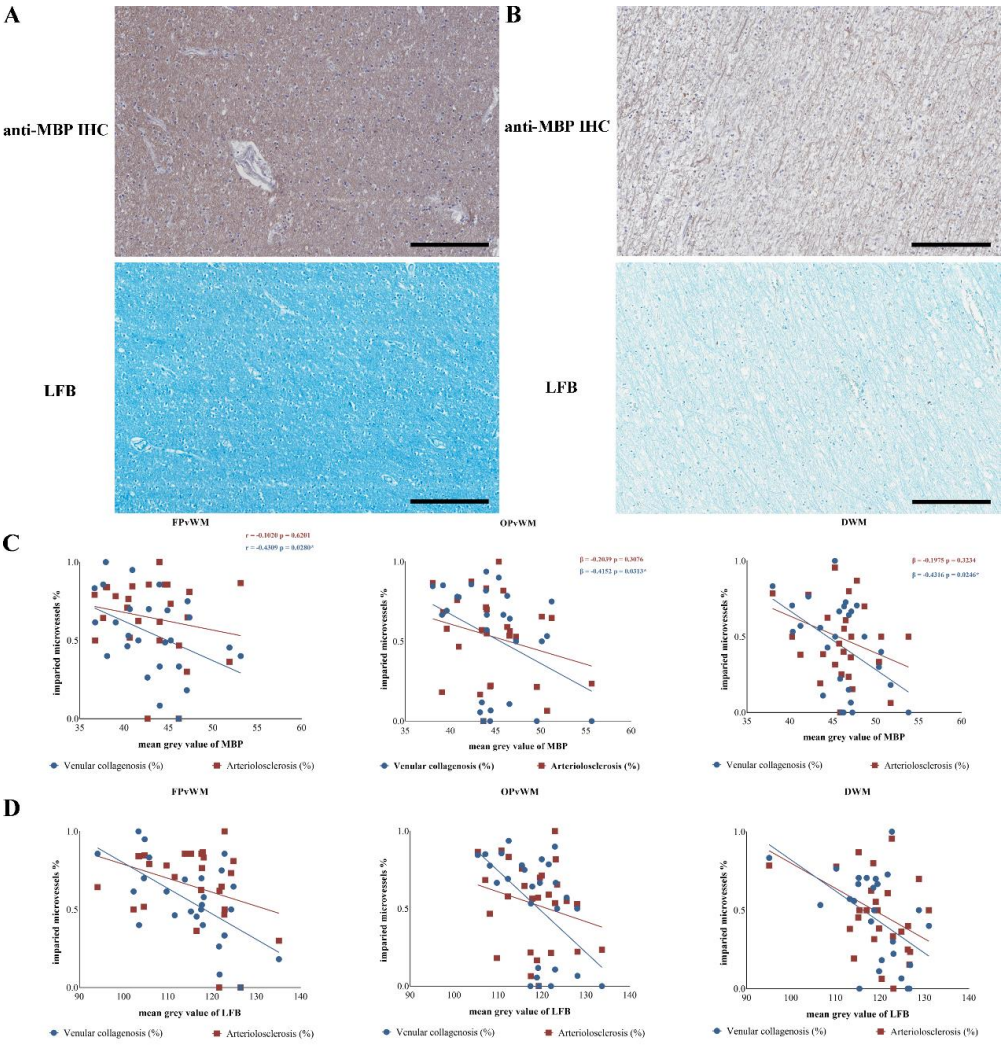
Supplementary Table 2. Primary antibodies and antigen recovery protocols

Steps	Contents
1	Pre-heat the Na-Citrate buffer (10 mM, pH 6.5, ZLI-9064, ZSGB-BIO) in the microwave. Cook the slides for 20 min in the microwave. The slides should never dry, so check every min and add Na-Citrate when necessary.
2	Let cool at room temperature.
3	Block endogenous peroxidase activity with 3% H_2O_2 (ZLI-9311, ZSGB-BIO) for 15 min.
4	Soak in PBS 3 x 3 min.
5	Block non-specific sites with 5% normal goat serum for 30 min (WGAR1009-5, Servicebio).
6	Incubate in primary antibodies (αSMA, Cat# 19245, RRID: AB_2734735, rabbit monoclonal antibody, diluted 1:400, Cell Signaling Technology; MCT1, Cat# 20139-1-AP, RRID: AB_2878645, rabbit polyclonal antibody, diluted 1:800, Proteintech) overnight at +4°C.
7	Let warm at room temperature.
8	Soak in PBS 3 x 3 min.
9	Incubate with the secondary antibody conjugated with biotin (Rabbit IgG, PK-4001, Vector Laboratories) for 30 min at room temperature. Prepare ABC reagent (Rabbit IgG, PK-4001, Vector Laboratories) simultaneously because it has to develop for at least 30 minutes before use.
10	Soak in PBS 3 x 3 min.
11	Incubate with ABC reagent for 30 min at room temperature.
12	Soak in PBS 3 x 3 min.
13	Prepare DAB peroxidase substrate (G1212-200T, Servicebio) immediately before use. Drop the DAB substrate on top of the slides and watch for brown staining.
14	Dip slides into tap water to stop the reaction.
15	Counterstain with Hematoxylin (2 min) and rinse in tap water until the water comes out clean.
16	Dehydrate through alcohol gradient starting at 70% ethanol up to 100% ethanol (5 min each).
17	Soak in xylene for 2 x 5 min.
18	Mount with neutral balsam mounting medium (ZLI-9555, ZSGB-BIO). Do not move the coverslip until completely dry, which takes ~ 24 hours.

Supplementary Table 3. Correlation of the burden of arteriosclerosis and venular collagenosis in brain regions

Brain region	severe arteriosclerosis and mild venular collagenosis n (%)	mild arteriosclerosis and severe venular collagenosis n (%)	both severe n (%)	both mild n (%)
FPvWM	5 (18.52)	4 (14.81)	9 (33.33)	9 (33.33)
OPvWM	3 (11.11)	1 (3.70)	10 (37.04)	13 (48.15)
DWM	2 (7.41)	5 (18.52)	8 (29.63)	12 (44.44)
SFC	4 (14.81)	2 (7.41)	10 (37.04)	11 (40.74)
OC	3 (11.11)	3 (11.11)	10 (37.04)	11 (40.74)
Hippocampus	3 (11.11)	3 (11.11)	10 (37.04)	11 (40.74)
Putamen	2 (7.41)	3 (11.11)	12 (44.44)	10 (37.04)

FPvWM = frontal periventricular white matter, OPvWM = occipital periventricular white matter, DWM = deep white matter of parietal lobe, SFC = superior frontal cortex, OC = occipital cortex. Cases were divided into mild and severe groups according to the median of total arteriolosclerosis or venular collagenosis burden.

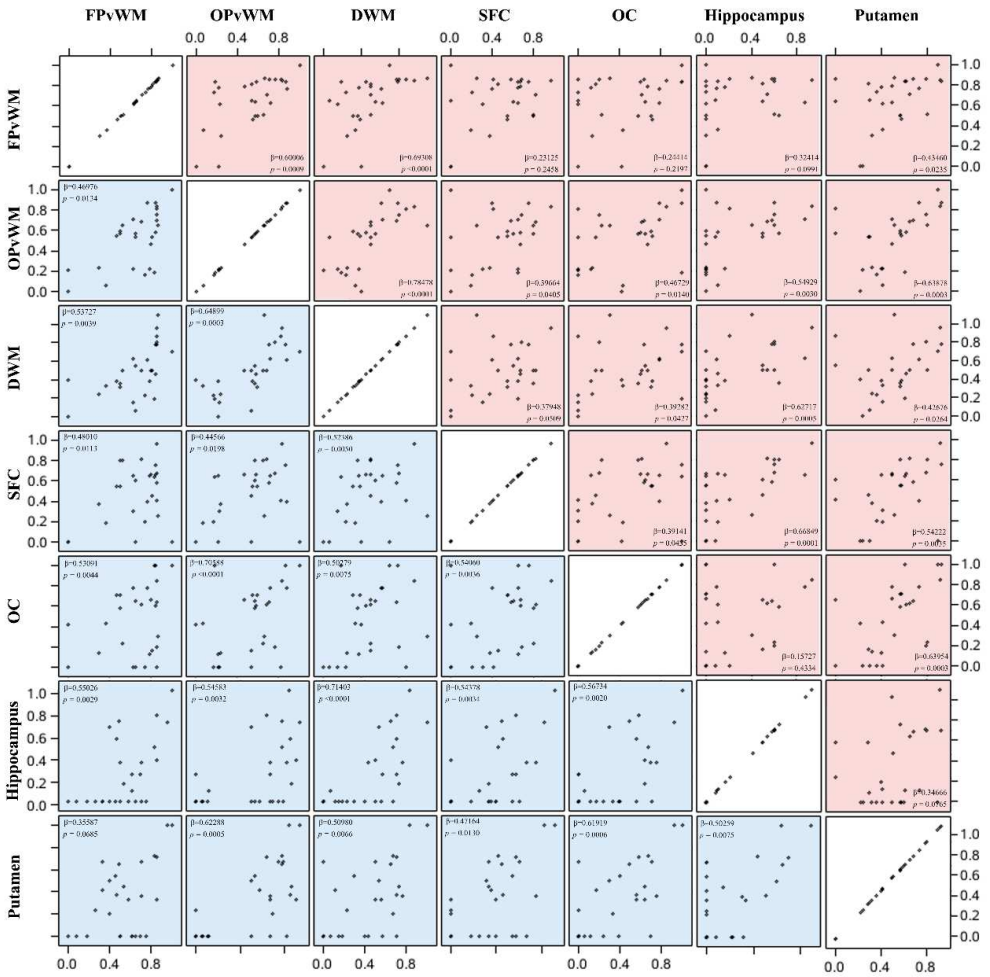


Supplementary Figure 1. Associations between myelin loss using various evaluation methods and arteriosclerosis, venular collagenosis in different brain regions.

Both Luxol fast blue (LFB) and anti-myelin basic protein (MBP) immunohistochemistry (IHC) stains were conducted to represent the severity of myelin loss in our study. Stains of Patient no.16 with relative mild myelin loss were shown in A, and those of Patient no.11 with severe myelin loss and brain atrophy were shown in B. As a common and convenient assessment method, LFB had a good consistency with anti-MBP IHC stain. Results of associations between myelin loss evaluated by LFB stain or anti-MBP IHC stain and arteriosclerosis, venular collagenosis in different white matter regions were concordant (C and D). No association between the degree of myelin loss and arteriolosclerosis was observed in both the periventricular and deep white matter; however, venular

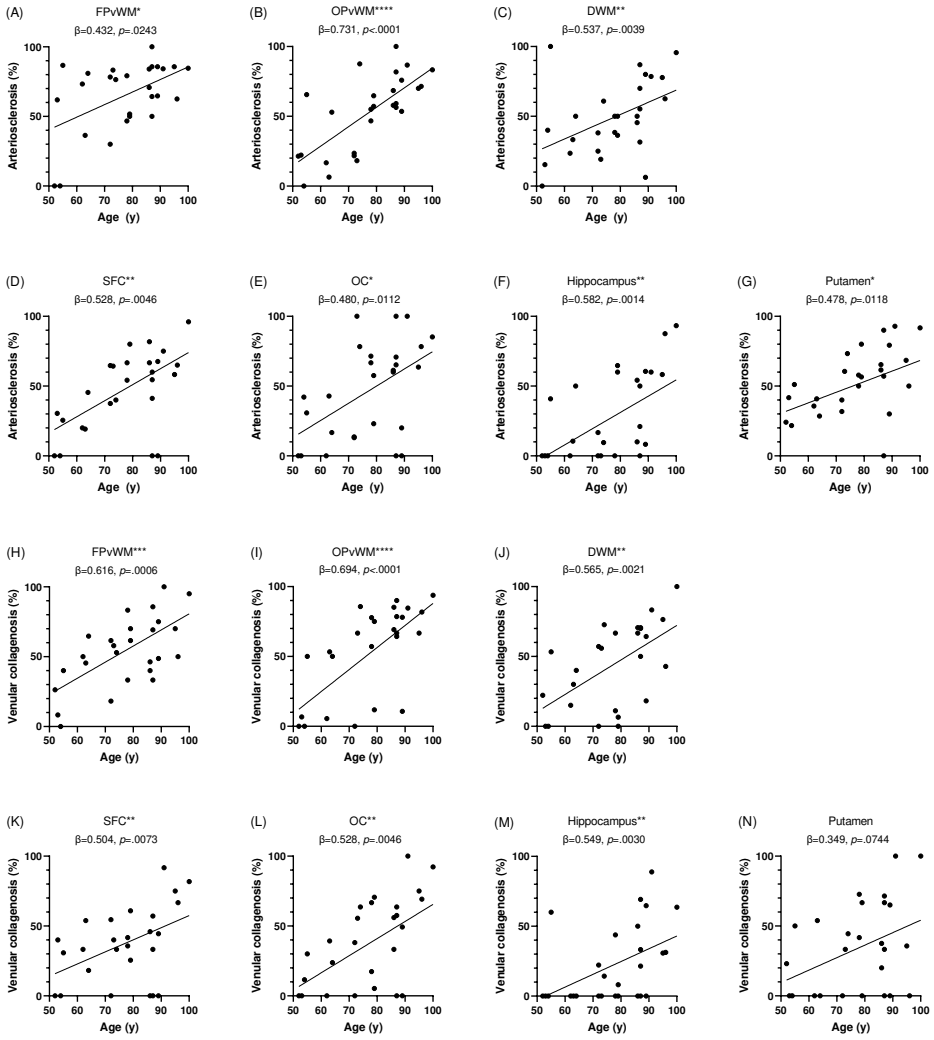
collagenosis was associated with white matter pallor in the occipital periventricular white matter and deep white matter. Bar = 200 μm for A ~ D.

LFB = Luxol fast blue, MBP = myelin basic protein, IHC = immunohistochemistry, FPvWM = frontal periventricular white matter, OPvWM = occipital periventricular white matter, DWM = deep white matter of parietal lobe.



Supplementary Figure 2. The correlation of arteriosclerosis or venular collagenosis burden between each region

FPvWM = frontal periventricular white matter, OPvWM = occipital periventricular white matter, DWM = deep white matter of parietal lobe, SFC = superior frontal cortex, OC = occipital cortex. Red boxes represent arteriosclerosis, and blue boxes represent venular collagenosis. Numbers on the axes represent the ratio of arteriosclerosis or venular collagenosis burden in the region. Each box represents the correlation of the microvascular wall pathology between two specific brain regions. The correlation coefficient and p-value are shown in each box.



Supplementary Figure 3. Arteriosclerosis and venular collagenosis increased with age

Data are shown as scatterplots.

FPvWM = frontal periventricular white matter, OPvWM = occipital periventricular white matter, DWM = deep white matter of parietal lobe, SFC = superior frontal cortex, OC = occipital cortex.

**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.