Cerebral hypoperfusion accelerates cerebral amyloid angiopathy and promotes cortical microinfarcts

Yoko Okamoto · Toru Yamamoto · Raj N. Kalaria · Hideto Senzaki · Takakuni Maki · Yoshiki Hase · Akihiro Kitamura · Kazuo Washida · Mahito Yamada · Hidefumi Ito · Hidekazu Tomimoto · Ryosuke Takahashi · Masafumi Ihara

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Abstract Cortical microinfarcts (CMIs) observed in brains of patients with Alzheimer’s disease tend to be located close to vessels afflicted with cerebral amyloid angiopathy (CAA). CMIs in Alzheimer’s disease are preferentially distributed in the arterial borderzone, an area most vulnerable to hypoperfusion. However, the causal association between CAA and CMIs remains to be elucidated. This study consists of two parts: (1) an observational study using postmortem human brains (n = 31) to determine the association between CAA and CMIs, and (2) an experimental study to determine whether hypoperfusion worsens CAA and induces CMIs in a CAA mouse model. In postmortem human brains, the density of CMIs was 0.113/cm² in mild, 0.584/cm² in moderate, and 4.370/cm² in severe CAA groups with a positive linear correlation (r = 0.6736, p < 0.0001). Multivariate analysis revealed that, among seven variables (age, disease, senile plaques, neurofibrillary tangles, CAA, atherosclerosis and white matter damage), only the severity of CAA was a significant multivariate predictor of CMIs (p = 0.0022). Consistent with the data from human brains, CAA model mice following chronic cerebral hypoperfusion due to bilateral common carotid artery stenosis induced with 0.18-mm diameter microcoils showed accelerated deposition of leptomeningeal amyloid β (Aβ) with a subset of them developing microinfarcts. In contrast, the CAA mice without hypoperfusion exhibited very few leptomeningeal Aβ depositions and no microinfarcts by 32 weeks of age. Following 12 weeks of hypoperfusion, cerebral blood flow decreased by 26% in CAA mice and by 15% in wild-type mice, suggesting impaired microvascular function due to perivascular Aβ accumulation after hypoperfusion. Our results suggest that cerebral hypoperfusion accelerates CAA, and thus promotes CMIs.

Keywords Cerebral amyloid angiopathy · Cortical microinfarcts · Tg-SwDI · Bilateral common carotid artery stenosis

Introduction

Cortical microinfarcts (CMIs) are frequently observed in the brains of Alzheimer’s disease (AD) patients [33, 41], and tend to be located in the vascular territory of leptomeningeal arteries or cortical arterioles exhibiting cerebral amyloid angiopathy (CAA), a pathological hallmark of AD [20, 33]. Furthermore, CMIs in AD are preferentially distributed in the arterial borderzone, an area particularly vulnerable to hypoperfusion [41], suggesting a causal relationship between CAA and CMIs, with hypoperfusion...
serving as a mediating factor. Indeed, our neuropathological study has shown that CMIs are present predominantly in the arterial borderzone between the middle and posterior cerebral artery territories in AD patients [33], which may coincide with the relative predilection of CAA pathology in posterior brain regions [42].

Nevertheless, the previous reports on the association between CAA and CMIs in AD brains have been conflicting [12, 24, 34, 40]; some studies have reported an association [34, 40] while others have found no such link [12, 24] (Supplementary Table 1). One of the plausible explanations for the disparity in the previous reports is the difficulty in assessing how the various AD-related pathologies, such as senile plaques (SPs), neurofibrillary tangles (NFTs), and CAA contribute to CMI development. Another complication arising from the findings is that these pathological changes can also be interdependent on concomitant atherosclerosis or arteriolosclerosis [34, 46]. Such difficulties are exemplified by the fact that SPs and CAA appear in close proximity in AD patients [48], though on a case-by-case level, an inverse correlation between CAA and plaque density is apparent [50].

The purpose of this study was to elucidate the possible association between the burden of CMIs and CAA by investigating postmortem brains exhibiting CAA where the final neuropathological diagnoses included not only AD but also other neurodegenerative disorders and vascular cognitive impairment. By avoiding a biased selection of postmortem brains, we anticipated this study would enable comparison of AD and non-AD patients that are accompanied by pathologically proven CAA to investigate the association between CMIs and CAA.

To explore whether chronic cerebral hypoperfusion is associated with the relationship between CMIs and CAA, we used a transgenic mouse CAA model that expresses human vasculotropic Swedish/Dutch/Iowa mutant amyloid precursor protein (APP) (Tg-SwDI mice) [11] and subjected them to bilateral common carotid artery stenosis (BCAS) to mimic chronic cerebral hypoperfusion [37]. By combining the animal investigations with postmortem human work, we aimed to reveal the underlying mechanisms in the relationship between CMIs and CAA.

Materials and methods

Postmortem human brain material

Two hundred seventy-five autopsied brains were obtained from Kyoto University Hospital and Osaka Saiseikai Nakatsu Hospital from 1992 to 2009 through a process approved by an institutional research committee. As we described previously [1, 21], neuropathological diagnoses were made by thorough histopathological examination of extensively sampled brain sections (Supplementary Fig. 1). In brief, in all brains at least 20 different samples, including anteroindorferter frontal region, anterior cingulate region, middle frontal gyrus, superior frontal gyrus, precentral gyrus, superior and middle temporal gyri, amygdala, hippocampus, entorhinal cortex, supramarginal sulcus, occipital lobe, basal ganglia, thalamus, cerebellum, and at least 3 levels of the brainstem, were systematically taken from the formalin-fixed brains (Supplementary Fig. 1).

Among the 275 patients, 31 patients were pathologically proved to have CAA by hematoxylin and eosin (H&E) staining and then by β-amyloid (Aβ) immunostaining, all of which were included in this study. Autopsies were performed at 12.9 ± 11.9 h (mean ± SD) (range 1.5–45.5 h) after death. The average fixation time was 37 ± 57 days (range 6–330 days) (Supplementary Table 2).

Clinical and pathological diagnosis

The 31 patients consisted of 14 AD (mean ± SD, 81 ± 8-year old) and 17 non-AD patients (78 ± 8-year old). The breakdown of the 17 non-AD patients is listed in Supplementary Table 2. The premortem clinical diagnoses, causes of death, vascular risk factors, postmortem pathological diagnoses and other demographic and pathological data of the 31 patients are also shown in Supplementary Table 2.

The clinical diagnosis of dementia met the criteria of the Diagnostic and Statistical Manual of Mental Disorders IV [3]. The neuropathological diagnoses of AD were made if the postmortem brains revealed the presence of frequent neuritic plaques in the neocortex (Consortium to Establish a Registry for Alzheimer’s Disease, CERAD) [29], and NFT stage was no less than IV, according to the Braak and Braak neuropathological staging of Alzheimer-related changes [7, 8] as assessed with modified Bielschowsky staining. Two observers (T.Y. and Y.O.) assessed SP and NFT stage individually, and if required, a joint assessment was scrutinized under a two headed microscope. The diagnosis of diffuse Lewy body disease was also made by thorough histopathological examination of extensively sampled brain sections [27]. The diagnosis of subcortical ischemic vascular dementia was made clinically [5], and was retrospectively found to meet the pathological criteria outlined by Kalaria et al. [19]: (1) the presence of bilateral diffuse white matter lesions, (2) the presence of lacunar infarctions in the perforator territory, and (3) the presence of arteriolosclerosis.

Grading of atherosclerosis

At autopsy, the atherosclerosis stage was consistently graded by one of the authors (T.Y.). The degree of atherosclerosis at...
skull base was classified into four grades: ‘normal’ (no ath-
erosclerosis), ‘mild’ (the presence of patchy atheroma),
‘moderate’ (a severity that is intermediate between mild and
severe), or ‘severe’ (the presence of atheroma along the
entire length of the vessels). The above staging was
re-examined and confirmed by another author (Y.O.) using
the autopsy report and macroscopic images taken at autopsy.

Tinctorial and immunohistochemical staining

Tissue blocks were obtained from the frontal, temporal,
parietal, and occipital lobes (Supplementary Fig. 1). The
blocks were embedded in paraffin and sectioned at 12 µm
thickness for Congo Red staining, and 6 µm thickness for
other staining on a microtome. To minimize variability in
staining intensity, tissue sections were prepared by the same
technician and stained with or using freshly prepared tinctorial
and buffer solutions. Routine histological assessment
was carried out with Congo Red, H&E, Klu¨ver-Barrera
(KB), modified Bielschowsky, and Gallyas staining. Pearls-
Stieda staining was added as it was needed. The rest of the
blocks were used for immunohistochemistry, involving
sequential incubation with primary antibody, appropriate
biotinylated secondary antibody (diluted 1:200, Vector
Laboratories, Burlingame, CA, USA), and avidin–biotin–
biotinylated secondary antibody (diluted 1:200, Vector
Laboratories). The primary antibodies were mouse anti-Aβ
(1:100, Novo- castra, Newcastle, UK), rabbit anti-cow glial fibrillary acidic
protein (GFAP) (1:200, DAKO, Glostrup, Denmark), mouse
anti-human paired helical filament-tau (AT8; 1:200, Thermo
Scientific, Rockford, IL, USA), and mouse anti-cluster of
differentiation 68 (CD68) (1:100, DAKO) antibodies.

Senile plaque and neurofibrillary tangle burden

The burden of neuritic plaques was classified into ‘none’,
‘sparse’, ‘moderate’, and ‘frequent’ categories in the cortical
sections stained with the modified Bielschowsky staining
according to CERAD protocol [29]. The stage of NFTs was
assessed according to the Braak and Braak neuropathological
staging of Alzheimer-related changes [7, 8]. In this study, we
used 6 µm thick paraffin sections using the modified Biels-
chowsky method as we have previously reported [52];
because, the modified Bielschowsky method is far more
effective over other silver staining methods in detecting NFTs.

Staging of CAA

For staging of CAA, 12 µm thick sections were stained
with Congo Red and viewed with polarized light [13, 42].
CAA was classified into CAA Type 1 (affected capillaries
with or without larger cerebral vessel involvement) and
CAA Type 2 (affected leptomeningeal arteries, cortical
arteries/arterioles, or rarely veins), as proposed by Attems
et al. [4]. CAA Type 2 was further analyzed, and was
divided into three grades proposed by Vonsattel et al. [44]:
those with ‘mild’ (focal Aβ deposits in the smooth muscle
layer of the vessel walls), ‘moderate’ (circumferential Aβ
deposits in the smooth muscle layer of the vessel walls),
and ‘severe’ (extensive Aβ deposition with morphological
changes such as microaneurysms, fibrinoid necrosis, dou-
ble barreling, inflammation, thrombus, or hemorrhage)
(Fig. 1). When several grades were observed in one case,
the dominant grade represented the case.

Definition and quantitative analysis of CMI

CMIs were analyzed in the same sections that were used
for pathological confirmation of CAA. CMIs were defined
as cerebral cortex lesions visible only microscopically [19]
and usually accompanied by reactive glial proliferation.
Regions of interest with evidence of expanded Virchow-
Robin space or microabscess as well as those accompanied
by hemorrhagic changes or cortical laminar necrosis on
H&E staining were excluded from analysis. Following
further confirmation of CMIs with immunohistochemistry
for GFAP and CD68, we determined the density of CMIs
using a method reported previously [33]. In brief, the
number of CMIs in each lobe was counted in sections
stained with H&E. Images of the H&E stained slides were
scanned (GT-X770 EPSON, Nagano, Japan). The cerebral
cortices were outlined on each slide and the areas were
measured using the ImageJ software package (image pro-
cessing and analysis in JAVA, ImageJ bundled with JAVA
1.43, NIH, USA). The number of CMIs per cm2 of the
cortex was calculated as a measure of CMI density.
The CMI density in the frontal cortex was the mean of the
values obtained from the five areas (anteroinferior frontal
region, anterior cingulate region, middle frontal gyrus,
superior frontal gyrus, precentral gyrus), and that in the
temporal cortex was the mean of the values obtained from
the two areas (lateral and medial temporal). The CMI
density in the parietal cortex was obtained from the parietal
supramarginal gyrus, and that in the occipital cortex was
obtained from the occipital calcarine cortex (Supplemen-
tary Fig. 1).

Assessment of white matter changes

Using H&E- and KB-stained slides cut coronally at the
level of mid-hippocampus, parietal, and occipital lobes, we
classified white matter lesions into four grades as reported
previously [9, 17]: those with ‘normal’ (normal white

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matter), ‘mild’ (no appreciable reduction in axonal meshwork density, and a slightly increased number of reactive astrocytes), ‘moderate’ (a slight reduction of axonal meshwork density, a reduction of oligodendroglial cell nuclei, and a further increased number of reactive astrocytes), and ‘severe’ (a marked reduction of myelin, axons and oligodendroglial cell nuclei with relatively marked astrocytic reaction, loosely scattered macrophages but no complete cerebral infarction).

Experimental animals

We used transgenic mice, C57BL/6-Tg(Thy1-APPSwDu-tIowa)BWevn/J [11] (Jackson Laboratory, Bar Harbor, ME, USA), which overexpress the neuronally derived human APP gene, encoding the Swedish p.K670N/M671L, Dutch p.E693Q and Iowa p.D694N mutations, under the control of the mouse thymus cell antigen 1 (Thy1) promoter. Generally, Thy1-driven exogenous gene expression is not altered by hypoxic/ischemic condition [26, 43]. The mice were screened for transgene expression by polymerase chain reaction, and heterozygous mice were mated with non-transgenic C57BL/6J mice (Japan SLC, Hamamatsu, Japan). All mice were given free access to food and water.

Surgical procedures and rearing methods

Male heterozygous mice were subjected to either sham or BCAS operation using microcoils [22, 30, 32, 37, 38]. Body weight and rectal temperature were measured, and blood pressure was monitored preoperatively and postoperatively at 32 weeks of age (12 weeks after BCAS or sham operation). Mean value of ten replicate measurements of CBF or SBP was determined for each mouse. The SBP was monitored in conscious mice by the tail-cuff method (MK-2000ST; Muromachi Co., Kyoto, Japan). The CBF was measured in identically sized regions of interest (900 pixels) located 1 mm posterior and 2 mm lateral from the bregma by Laser speckle blood flow imager (Omega Zone; Omegawave, Tokyo, Japan) under anesthesia with 1.5% isoflurane after the periosteum was widely removed with fine-tip forceps and calibration was carried out with a calibration reference device (Calibrator S/N 080715-5, Omegawave, Inc., Tokyo, Japan). CBF values were expressed as a percentage of the preoperative value.

Fig. 1 Representative photomicrographs of various grades of CAA. Congo Red staining showing mild CAA (a), moderate CAA (b), and severe CAA associated with double barreling (c). Bars indicate 100 μm in a and c, and 50 μm in b.

Systolic blood pressure and cerebral blood flow measurements

Mice were thermostatically controlled at 37°C on a warming pad and cerebral blood flow (CBF) and systolic blood pressure (SBP) recorded preoperatively and postoperatively at 32 weeks of age (12 weeks after BCAS or sham operation). Mean value of ten replicate measurements of CBF or SBP was determined for each mouse. The SBP was monitored in conscious mice by the tail-cuff method (MK-2000ST; Muromachi Co., Kyoto, Japan). The CBF was measured in identically sized regions of interest (900 pixels) located 1 mm posterior and 2 mm lateral from the bregma by Laser speckle blood flow imager (Omega Zone; Omegawave, Tokyo, Japan) under anesthesia with 1.5% isoflurane after the periosteum was widely removed with fine-tip forceps and calibration was carried out with a calibration reference device (Calibrator S/N 080715-5, Omegawave, Inc., Tokyo, Japan). CBF values were expressed as a percentage of the preoperative value.
repetitive CBF measurement leads to fibrous scar tissue build up and bone opacification, CBF was measured only at two time points.

Histological investigation in mice

Mice were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal) and transcardially perfused with 0.01 M phosphate buffer (PB) in normal saline. The removed brains were immersion-fixed in 4% paraformaldehyde in 0.1 M PB, and embedded in paraffin. The brains were then sliced into 6 μm thick sagittal sections at 1, 2, 3, and 4 mm lateral from the midline, and subjected to H&E and modified Bielschowsky staining. Immunohistochemical staining was performed according to the same protocol as human tissue. Mouse anti-Aβ1–40 (BA27) (Wako amyloid kit, Wako, Osaka, Japan), mouse anti-Aβ1–42 (BC05) (Wako amyloid kit, Wako), and mouse anti-Aβ1–40 (6E10) (1:500, Covance, Princeton, NJ, USA) antibodies were used.

Densitometric analysis of mouse brains

The Aβ-stained slides were captured with a digital camera (BZ-9000 KEYENCE, Osaka, Japan). Then, using the ImageJ software, the densitometric analysis of Aβ was performed blindly to animal groups by setting regions of interest in the cerebral cortex, the hippocampus, and the leptomeninges with the identical threshold in the Aβ (6E10)-immunostained sections. Leptomeningeal vascular, as well as pial, Aβ accumulations were jointly analyzed as ‘leptomeningeal Aβ’.

Statistical analysis

For human samples, numerical scores were computed from the data analysis as follows: age, 65- to 93-year old; disease group, AD = 1 or non-AD = 0; the grade of atherosclerosis, 0–3; the severity of CAA, 1–3; SP burden, 0–3; NFT stage, 0–6; and the grade of WML, 0–3. We first performed univariate analysis to determine whether age, disease type, or the above pathological changes were predictive of CMI formation using Fisher’s exact test. Next, multivariate analysis was performed while taking into account the effects of all variables on the parameters measured, including CMI formation.

In mice, Student’s t test was used to evaluate possible differences between the sham- and BCAS-operated mice groups at each time point, and two-way ANOVA was used to test for the effect of age and operation on Aβ deposition in the hippocampus, cerebral cortex, and leptomeninges. Differences with p < 0.05 were considered statistically significant in all analyses used.

Results

The grade of CAA and atherosclerosis, SP and NFT stage, the degree of WML, and the CMI densities in the autopsy series are shown in Table 1.

Capillary CAA

Capillary CAA was found in only one patient (Table 1, Patient no. 26). In this patient, findings of Aβ depositions in the leptomeningeal arteries and cortical arterioles were also prominent. In the remaining 30 patients, capillary CAA was undetectable with H&E staining and Aβ immunohistochemistry. In the following analyses, therefore, only CAA Type 2 was graded.

Cortical microinfarcts

CMIIs were present along the sulcus in the superficial cortex with variable frequency among the postmortem brains (Fig. 2a, b). The average microinfarct diameter was approximately 200 μm (range 100–500 μm). Some lesions were recently formed, with the presence of GFAP-positive gemistocytic astroglial cells and CD68-positive microglia/macrophages (Fig. 2c–e), while others were relatively old with induction of fibrillary astrocyte gliosis. Thrombus formation was found in one patient with severe CAA (Table 1, Patient no. 13).

Most CMIs (>90%) were located in close proximity (~1 cm) to Aβ-deposited vessels (Fig. 3a–c). Around ischemic foci, such as CMIs or necrosis, SPs were sometimes not present (Fig. 3c). Furthermore, in and around ischemic foci, vascular Aβ deposition appeared to increase in intensity (Fig. 3a–c).

CMI density in AD and non-AD patients

The results of the univariate analyses are shown in Table 2. The mean CMI density within the four cortical lobes was not significantly different (r = -0.1007, p = 0.5900) in AD group (0.71/cm²) and non-AD group (1.12/cm²) (Fig. 4a). When AD and non-AD groups were combined, CMI density in the frontal, temporal, parietal, and occipital lobes was 1.02, 0.65, 1.18, and 0.93/cm², respectively (Fig. 4b). There were no significant differences in the CMI density within the four cortical lobes. CMI density in the frontal, temporal, parietal, and occipital lobes was 1.32, 0.29, 1.03, and 0.30/cm², respectively, in the AD group, while in the non-AD group the CMI density was 0.76, 0.97, 1.29, and 1.44/cm², respectively (Fig. 4c).

CMI density was 0.11/cm² in mild, 0.58/cm² in moderate, and 4.37/cm² in severe CAA groups, meaning it became greater as CAA severity increased (r = 0.6736,
This CAA–CMI correlation was observed in all lobes (Fig. 4e); the \( r \) value was 0.6377 in the frontal lobe (\( p = 0.0014 \)), 0.5676 in the temporal lobe (\( p = 0.0055 \)), 0.5355 in the parietal lobe (\( p = 0.0078 \)), and 0.5652 in the occipital lobe (\( p = 0.0033 \)). Such CAA–CMI correlation was also observed in both disease groups (AD or non-AD) (Fig. 4f).

CMI density was not associated with SP burden (\( r = -0.2265, p = 0.2204 \)) (Fig. 4g), or NFT stage (\( r = -0.1378, p = 0.4597 \)) (Fig. 4h). Thus, CMI density did not change with greater burden of SP or NFT. CMI density was also not associated with the grade of atherosclerosis (\( r = -0.0913, p = 0.6312 \)) (Fig. 4i).

The results of the multivariate analysis are shown in Table 3. The severity of CAA was a significant multivariate predictor (\( p = 0.0022 \)), with a standard partial regression coefficient of 0.6395. Similar trends were evident in each lobe (frontal, \( p = 0.0014 \); temporal, \( p = 0.0055 \); parietal, \( p = 0.0078 \); occipital, \( p = 0.0033 \)). However, age (\( p = 0.5979 \)), the grade of atherosclerosis (\( p = 0.3973 \)), AD or non-AD (\( p = 0.8364 \)), SP burden (\( p = 0.5164 \)), NFT stage (\( p = 0.3870 \)), or the grade of WML (\( p = 0.6931 \)) were not significant multivariate predictors.

Cerebral blood flow in mice after BCAS

The BCAS operation reduced CBF by 15% in 20-week-old wild-type mice and 26% in Tg-SwDI mice of the same age after 12 weeks with a statistically significant difference (\( p = 0.0412 \)) (Supplementary Fig. 3).

| No. | Age | Gender | AD or non-AD | Grade of atherosclerosis | CAA | SP | NFT | CMI density (\(/ \text{cm}^2\)) | WML |
|-----|-----|--------|--------------|--------------------------|-----|----|-----|-------------------------------|-----|
| 1   | 85  | M      | AD           | Moderate                 | Mild | Frequent | IV | 0.00 | 0.00 | 0.00 | Mild |
| 2   | 82  | F      | AD           | Mild                     | Mild | Frequent | IV | 0.00 | 0.40 | 0.00 | Normal |
| 3   | 76  | F      | AD           | Mild                     | Mild | Frequent | IV | 0.51 | 0.00 | 0.00 | Moderate |
| 4   | 86  | F      | AD           | Mild                     | Mild | Frequent | IV | 0.00 | 0.26 | 0.47 | 0.00 | Mild |
| 5   | 80  | F      | AD           | Mild                     | Mild | Frequent | V  | 0.45 | 0.00 | 0.54 | 0.00 | Moderate |
| 6   | 67  | F      | AD           | Mild                     | Mild | Frequent | VI | 0.00 | 0.00 | 0.31 | 0.00 | Mild |
| 7   | 84  | F      | AD           | Mild                     | Mild | Frequent | VI | 0.09 | 0.00 | 0.00 | 0.00 | Normal |
| 8   | 68  | M      | AD           | Mild                     | Moderate | Frequent | IV | 0.00 | 0.00 | 0.00 | 0.00 | Mild |
| 9   | 93  | F      | AD           | Moderate                 | Moderate | Frequent | VI | 0.71 | 0.00 | 0.00 | 0.00 | Mild |
| 10  | 84  | F      | AD           | Moderate                 | Moderate | Frequent | V  | 0.00 | 0.26 | 0.00 | 0.00 | Moderate |
| 11  | 91  | M      | AD           | Mild                     | Moderate | Frequent | IV | 0.64 | 0.30 | 0.55 | 0.11 | Severe |
| 12  | 85  | F      | AD           | Moderate                 | Moderate | Frequent | V  | 1.55 | 0.55 | 7.01 | 0.00 | Mild |
| 13  | 83  | M      | AD           | Normal                   | Severe | Frequent | IV | 12.6 | 1.49 | 0.31 | 2.31 | Moderate |
| 14  | 71  | M      | AD           | Mild                     | Severe | Frequent | IV | 2.00 | 0.47 | 4.18 | 1.58 | Mild |
| 15  | 90  | M      | AD           | Mild                     | Mild | Sparse | IV | 0.00 | 0.00 | 0.00 | 0.00 | Moderate |
| 16  | 66  | M      | Non-AD       | Severe                   | Mild | Sparse | II | 0.00 | 0.00 | 0.00 | 0.29 | Normal |
| 17  | 83  | F      | Non-AD       | Mild                     | Mild | Sparse | IV | 0.00 | 0.00 | 0.24 | 0.00 | 0.00 | Moderate |
| 18  | 84  | M      | Non-AD       | Mild                     | Mild | Sparse | I  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Moderate |
| 19  | 76  | M      | Non-AD       | Mild                     | Mild | Sparse | I  | 0.45 | 0.00 | 0.00 | 0.00 | 0.00 | Mild |
| 20  | 90  | F      | Non-AD       | Mild                     | Mild | Moderate | IV | 0.11 | 0.00 | 0.93 | 0.31 | Mild |
| 21  | 86  | M      | Non-AD       | Mild                     | Mild | Moderate | IV | 0.00 | 0.24 | 0.23 | 0.00 | 0.00 | Moderate |
| 22  | 84  | F      | Non-AD       | Mild                     | Mild | Moderate | II | 0.00 | 0.00 | 0.29 | 0.50 | Mild |
| 23  | 65  | M      | Non-AD       | Normal                   | Mild | Frequent | V  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Normal |
| 24  | 81  | M      | Non-AD       | Mild                     | Mild | Frequent | V  | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | Moderate |
| 25  | 72  | M      | Non-AD       | Moderate                 | Moderate | Sparse | II | 0.00 | 1.45 | 0.32 | 0.00 | 0.00 | Moderate |
| 26  | 75  | M      | Non-AD       | Severe                   | Moderate | Sparse | II | 0.19 | 2.59 | 1.22 | 0.33 | Mild |
| 27  | 73  | F      | Non-AD       | Mild                     | Moderate | Sparse | II | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | Mild |
| 28  | 82  | M      | Non-AD       | Normal                   | Moderate | Moderate | II | 0.49 | 0.00 | 0.38 | 2.17 | Mild |
| 29  | 71  | M      | Non-AD       | Moderate                 | Severe | None | II | 2.55 | 2.65 | 3.56 | 0.94 | Mild |
| 30  | 72  | F      | Non-AD       | Mild                     | Severe | Sparse | II | 2.33 | 1.10 | 1.66 | 6.26 | Mild |
| 31  | 69  | F      | Non-AD       | Mild                     | Severe | Sparse | III | 6.48 | 8.48 | 12.8 | 13.7 | Moderate |
Histological findings in Tg-SwDI mice

We found that microinfarcts developed in a subset of BCAS-operated Tg-SwDI mice (Fig. 5). The percentage of BCAS-operated Tg-SwDI mice (n = 17) exhibiting microinfarcts was 33.3% (2 out of 6), 42.9% (3 out of 7), and 25.0% (1 out of 4) at 18, 24, and 32 weeks of age. However, microinfarcts were not found in sham-operated Tg-SwDI mice (n = 14) or BCAS-operated wild-type mice (n = 15) up to 32 weeks of age. The number of microinfarcts in BCAS-operated Tg-SwDI mice, sham-operated Tg-SwDI mice, and BCAS-operated wild-type mouse was listed in Supplementary Table 3. Immunohistochemical analysis showed that ischemic insults accelerated perivascular and leptomeningeal Aβ accumulations (Fig. 6a, b). Generally, the immunostained patterns of Aβ40 and Aβ42 were quite similar, although Aβ40 immunostaining was more intense than Aβ42, in accordance with a previous biochemical study [11]. Aβ accumulation was evident in and around microvessels as well as larger cerebral vessels (approximately 10–15 μm in diameter), in the leptomeningeal arteries of BCAS-operated Tg-SwDI mice at
18 weeks of age; however, Aβ accumulation in the leptomeningeal arteries were less apparent in sham-operated Tg-SwDI mice at 18 or 24 weeks of age (Fig. 6b). As a result, two-way ANOVA showed that BCAS had a significant $[F(1, 25) = 18.5, p = 0.0002]$ effect on leptomeningeal Aβ accumulation. However, there was no effect of age on leptomeningeal Aβ accumulation $[F(2, 25) = 1.73, p = 0.20]$ (Fig. 6f), suggesting a ceiling effect of age on leptomeningeal Aβ accumulation.

By contrast, in the hippocampus and the cerebral cortex, two-way ANOVA showed a significant effect of age [hippocampus, $F(2, 25) = 15.8, p < 0.0001$; cortex, $F(2, 25) = 7.02, p = 0.0038$] but no effect of the operation [hippocampus, $F(1, 25) = 2.79, p = 0.11$; cortex, $F(1, 25) = 0.73, p = 0.40$] on Aβ accumulation (Fig. 6a, d, e).

In all Tg-SwDI mice combined, the amount of leptomeningeal Aβ was significantly greater in mice with microinfarcts (3.4-fold), compared to those without microinfarcts ($p = 0.0004$). Most microinfarcts were seen in the cortex (Fig. 5b) and close to the Aβ-deposited vessels; however, some microinfarcts were also seen in the hippocampus CA1 area in the 18, 24, and 32-week-old BCAS-operated Tg-SwDI mice (Fig. 5d). A small number of microhemorrhages were also detected in the ventral part of the thalamus in the 32-week-old BCAS-operated Tg-SwDI mice (Fig. 5e, f), although such changes were not found in sham-operated Tg-SwDI mice or BCAS-operated wild-type mice up to 32 weeks of age (Fig. 5a, c). Thrombus formation was not apparent in any sections assessed.

**Discussion**

We found that CMI density is related to CAA severity, but not to SP or NFT burden severity, suggesting that the presence of Aβ causes loss of vascular autoregulation associated with rigidity of arterioles, leading to infarction in the territory of their branching vessels. Thrombus formation induced by endothelial damage in CAA may have also been involved in the CMI development although the thrombus
was found only in one patient with CAA but not in any Tg-SwDI mice. The severity of white matter changes was not associated with the CMI density, which may raise the possibility that the CMIs and white matter changes have different formation processes as white matter damage is reported to reflect a progressive microangiopathy due to CAA [10]. Furthermore, our study showed that CMI density is no greater in AD than in other disorders accompanied with CAA. Given that CMI is also a strong determinant for dementia [23, 49], the observed correlation between CMI and CAA strengthens the notion that, although underestimated, these two pathologies are important substrates of cognitive decline in the elderly [15, 16].

Previous studies have reported an association between CMI and CAA in AD [15, 16, 34, 40], but others have found no such link [12, 24]. Inconsistencies between different studies might be attributable to the heterogeneity of underlying vascular pathology in AD as cerebrovascular changes can occur concomitantly with AD pathology, and also because CAA may contribute to the development of various types of cerebrovascular diseases such as lobar hemorrhage, microbleeds, and white matter lesions [39]. The issue is further complicated by the fact that CAA usually accompanies AD, but does not exclusively result from AD. Thus, accordingly, a significant correlation has been reported between the severity of CAA and the amount of CMIs in only one other study using postmortem brains of vascular dementia [16]. The current study, which used an unbiased collection of brains including non-AD samples, confirms the previous report and suggests that CAA along with chronic cerebral hypoperfusion may give rise to CMIs, which appear to be true substrates of cognitive impairment [14]. This may explain the previous findings, which have indicated that severity of CAA is associated with increased frequency of antemortem cognitive decline [25, 31]. Our findings, which show a strong association between CMIs and CAA, are supported by additional animal studies using the Tg-SwDI mice subjected to chronic cerebral hypoperfusion; when combined these findings have demonstrated that cerebral hypoperfusion induces: (1) accumulation of CAA-like vascular Aβ and resultant vascular compromise and (2) development of microinfarcts and even microhemorrhages in a subset of animals. Since microinfarcts did not develop in wild-type mice after BCAS, the data suggest that both CAA and hypoperfusion are required for microinfarct development. Tg-SwDI mice may have morphological changes in the endothelium and in the basement membranes of capillaries and arteries, leading to a failure of the mechanisms of clearance of Aβ and other metabolites. Hypoperfusion may interfere with the arterial pulsations and with the interstitial fluid pressure, leading to reduced perivascular clearance of Aβ as manifested by CAA associated with microinfarcts.

We have previously shown that chronic cerebral hypoperfusion increased the amount of filter-trap Aβ in the extracellular-enriched soluble brain fraction of mice over-expressing a mutant form of the human APP [22, 51]; in this mouse model exhibiting senile plaques, the neuronal loss and cognitive impairment that were both accelerated by hypoperfusion was explained by the disturbed Aβ metabolism. In the current study using mice overexpressing vasculotropic mutant Aβ, the hypoperfusion-induced aggravation of CAA may then result from increased synthesis of Aβ [45]. Alternatively, cerebral hypoperfusion and reduced vascular pulsation may impede clearance of Aβ as it is hypothesized that motive force of vessel pulsation is required for perivascular clearance of interstitial fluid containing Aβ [47]. This mechanism may temporarily

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### Table 2: Univariate analysis of variables

| Factors        | N  | CMI density | r value | p value |
|----------------|----|-------------|---------|---------|
| Age (years)    |    |             | -0.2570 | 0.1627  |
| <70            | 5  | 2.101       |         |         |
| 70–79          | 8  | 1.142       |         |         |
| 80–89          | 18 | 0.520       |         |         |
| Atherosclerosis|    |             | -0.0913 | 0.6312  |
| Normal (0)     | 3  | 1.642       |         |         |
| Mild (1)       | 19 | 0.916       |         |         |
| Moderate (2)   | 6  | 0.898       |         |         |
| Severe (3)     | 2  | 0.578       |         |         |
| Disease        |    |             | -0.1007 | 0.5900  |
| Non-AD (0)     | 17 | 1.118       |         |         |
| AD (1)         | 14 | 0.714       |         |         |
| CAA            |    |             | 0.6736  | <0.0001 |
| Mild (1)       | 17 | 0.112       |         |         |
| Moderate (2)   | 9  | 0.584       |         |         |
| Severe (3)     | 5  | 4.368       |         |         |
| SP             |    |             | -0.2265 | 0.2204  |
| None (0)       | 1  | 2.425       |         |         |
| Sparse (1)     | 10 | 1.511       |         |         |
| Moderate (2)   | 4  | 0.353       |         |         |
| Frequent (3)   | 16 | 0.628       |         |         |
| NFT            |    |             | -0.1378 | 0.4597  |
| I (1)          | 2  | 0.086       |         |         |
| II (2)         | 8  | 0.983       |         |         |
| III (3)        | 1  | 10.353      |         |         |
| IV (4)         | 12 | 0.641       |         |         |
| V (5)          | 5  | 0.528       |         |         |
| VI (6)         | 3  | 0.093       |         |         |
| WML            |    |             | 0.1962  | 0.2902  |
| Normal (0)     | 4  | 0.074       |         |         |
| Mild (1)       | 16 | 0.790       |         |         |
| Moderate (2)   | 10 | 1.567       |         |         |
| Severe (3)     | 1  | 0.400       |         |         |
Fig. 4 Correlation of CMI density with CAA severity. a A diagram showing no significant difference in CMI density between AD and non-AD groups (*p = 0.5900). b A diagram showing CMI density in each lobe of AD and non-AD groups combined. c Diagram showing CMI density in each lobe of AD and non-AD groups. d Significant correlation of CMI density with CAA severity (*p < 0.0001). e CAA-CMI correlation was observed in each lobe. f CAA-CMI correlation was observed in both AD and non-AD group. g CMI density was not associated with SP burden (*p = 0.2204). h CMI density was not associated with NFT stage (*p = 0.4597). i CMI density was not associated with atherosclerosis grade (*p = 0.6312)

Table 3 Multivariate analysis of variables

| Variables              | Partial regression coefficient | Standard partial regression coefficient | t value | p value | Tolerance |
|------------------------|--------------------------------|----------------------------------------|---------|---------|-----------|
| CAA severity           | 1.7190                         | 0.6395                                 | 3.4654  | 0.0022  | 0.4220    |
| NFT stage              | 0.3019                         | 0.2168                                 | 0.8825  | 0.3870  | 0.3555    |
| Atherosclerosis grade  | -0.4188                        | -0.1483                                | -0.8632 | 0.3973  | 0.7284    |
| SP burden              | -0.4848                        | -0.2346                                | -0.6596 | 0.5164  | 0.1723    |
| Age                    | -0.0244                        | -0.0968                                | -0.5352 | 0.5979  | 0.6704    |
| WML grade              | 0.1970                         | 0.0698                                 | 0.3999  | 0.6931  | 0.7247    |
| Disease                | -0.2555                        | -0.0626                                | -0.2090 | 0.8364  | 0.2468    |
aggravate CAA, which may compromise cerebral perfusion and ultimately lead to development of microinfarcts. Thus, the impaired microvascular function observed may impede perivascular drainage pathway of Aβ, forming a cycle of vascular Aβ deposition.

Our findings may also have implications in the understanding of the pathological complications of Aβ immunotherapy. The hypoperfusion-induced abnormalities seen in Tg-SwDI mice may be similar to the findings in AD patients who have received Aβ immunotherapy; in immunized AD group, microvascular lesions, such as CMIs or microhemorrhages, occurred with a higher density than non-immunized AD control group; this may have been because soluble Aβ mobilized from the amyloid deposits had not cleared efficiently from AD brains [6, 35]. It is plausible that the aging- and AD-associated microvascular changes [18] cannot cope with overwhelming amount of Aβ mobilized from the Aβ deposits at least partially due to existing reduction of vessel pulsation and/or hypoperfusion, leading to formation of CMIs or microhemorrhages in immunized AD patients. The current study indicates that such mechanisms underlie the formation of CMIs, even in...
non-immunized patients with CAA. The degree of hypoperfusion and microvascular changes may predict the response rate of Aβ immunotherapy and the emergence rate of ischemic complications. However, it remains to be determined whether chronic cerebral hypoperfusion hampers the Aβ drainage pathway directly through reduced arterial pulsation or indirectly through upregulated Aβ synthesis.

The main limitation of this study is the lack of clinical and neuropsychometric information on the postmortem brains used. This meant that we could not directly relate our pathological findings to antemortem hypoperfusive and cognitive status. Another limitation is that CAA or CMI severity could not be related to the apolipoprotein E genotype [36], due to ethical and legal constraints of genotype extraction. In addition, the use of the CERAD
criteria for assessing the burden of neuritic plaques and the Braak staging for the burden of NFTs both based on the modified Bielschowsky staining may be the third limitation, considering that the CERAD is semiquantitative rather than quantitative measures of neuropathology [28] and the Braak staging criteria for tau pathology have been recently modified [2], although the two neuropathologists agreed on their final grading of the 31 CAA patients enrolled in the current study.

In summary, using postmortem brain analysis of relevant patients and models of transgenic mice, our study provides strong evidence for the relationship between CAA and microinfarcts with cerebral hypoperfusion as the mediating factor. Our observations support the notion that CAA is one of the pathological substrates that links neurodegenerative and vascular processes.

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