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## High-definition characterization of cerebral $\beta$ -amyloid angiopathy in Alzheimer's disease

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### Abstract

The occurrence and progression of cerebral  $\beta$ -amyloid angiopathy (CAA) and  $\beta$ -amyloid plaques in sporadic Alzheimer's disease may be attributed to aging-related deficiencies in  $\beta$ -amyloid drainage along cerebral perivascular pathways. To elucidate high-definition characteristics of cerebral  $\beta$ -amyloid deposition, we performed immunogold silver staining for  $\beta$ -amyloid-40 and  $\beta$ -amyloid-42 on semithin LR White-embedded tissue sections from 7 Alzheimer's disease/severe CAA, 9 Alzheimer's disease/mild CAA, 5 old control and 4 young control autopsy brains. In vessel walls,  $\beta$ -amyloid-40 and  $\beta$ -amyloid-42 deposits were unevenly distributed along the adventitia and among the medial smooth muscle cells.  $\beta$ -Amyloid-40 immunoreactivity appeared greater than that of  $\beta$ -amyloid-42 in vessel walls, with  $\beta$ -amyloid-42 being preferentially located on their abluminal regions. In capillary walls, either  $\beta$ -amyloid-40 or  $\beta$ -amyloid-42 deposits or both were present in 6 of 7 severe CAA and 1 of 9 mild CAA cases, with a marked variation in thickness and focally abluminal excrescences. In 5 of 7 severe CAA cases, a subset of  $\beta$ -amyloid-laden capillaries revealed either  $\beta$ -amyloid-40 or  $\beta$ -amyloid-42 deposits or both radiating from their walls into the surrounding neuropil ("pericapillary deposits"). No vascular  $\beta$ -amyloid-40 or  $\beta$ -amyloid-42 deposits were observed in any of the controls. In conclusion, the patterns of  $\beta$ -amyloid-42 and  $\beta$ -amyloid-40 immunoreactivity in vessel walls suggest that  $\beta$ -amyloid deposits occur in the vascular basement membranes along cerebral perivascular drainage pathways, extending from cortical capillaries to leptomeningeal arteries. The presence of pericapillary  $\beta$ -amyloid deposits suggests that a subset of  $\beta$ -amyloid plaques originate from  $\beta$ -amyloid-laden capillaries, particularly in Alzheimer's disease brains that exhibit preferential capillary CAA involvement.

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## Keywords

Cerebral amyloid angiopathy; LR White resin; Immunogold silver staining

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## 1. Introduction

In sporadic late-onset Alzheimer's disease (AD) the neuropathologic characteristics,  $\beta$ -amyloid ( $A\beta$ ) plaques and hyperphosphorylated-tau neurofibrillary changes in brain parenchyma, are commonly accompanied by cerebral  $A\beta$  angiopathy (CAA) [1]. Amyloid deposits in AD brains are mainly composed of  $A\beta$  peptides 1-40 and 1-42 [2].  $A\beta_{42}$  is much more fibrillogenic than  $A\beta_{40}$ , and the  $A\beta_{40/42}$  ratio predicts their distribution in vessel walls and parenchymal plaques, whereby an increased  $A\beta_{40/42}$  ratio is associated with the development of CAA [2]. The apolipoprotein E  $\epsilon 4$  allelic dosage was shown to correlate with  $A\beta_{40}$  accumulation in parenchymal plaques [3,4] and vessel walls [5] previously seeded with  $A\beta_{42}$  in AD brains. Nonetheless, the mechanistic cascade of cerebral  $A\beta$  deposition is still unclear. In aging and sporadic AD there is no consistent evidence for either overproduction or abnormal isoforms of  $A\beta$ , in contrast to Down's syndrome and familial forms of AD [2]. Progressive cerebral  $A\beta$  accumulation may be associated with decreased enzymatic degradation of  $A\beta$  originating from neurons, deficient efflux of soluble  $A\beta$  from the interstitial fluid, increased influx of  $A\beta$  from the blood circulation, or a combination of these and other factors [6]. In addition to receptor-mediated transcytosis of  $A\beta$  across the blood-brain barrier,  $A\beta$  elimination may be mediated by perivascular macrophages [7], via bulk flow of interstitial fluid into the ventricles [8], and through perivascular interstitial fluid drainage of soluble  $A\beta$  along the basement membranes of capillaries and arteries [6,9].

In histologic preparations, insoluble or fibrillar  $A\beta$  deposits can be visualized in correlation with tissue morphology. Previous immunohistochemical studies of cerebral  $A\beta$  deposition were performed mostly on paraffin-embedded tissue sections [5,10-13], and occasionally using immunoelectron microscopy [14-16]. Immunohistologic evaluation on paraffin sections is likely restricted by relatively low definition. While ultrastructural visualization of protein markers of interest can be achieved by immunoelectron microscopy, the field size of examination is very limited with this approach. Immunogold silver staining of semithin resin-embedded tissue sections is considered a "compromise" method that provides higher-definition immunohistologic observations compared with those of paraffin immunohistochemistry, and larger field sizes compared to those that can be visualized by immunoelectron microscopy.

The present study was aimed at elucidating high-definition characteristics of  $A\beta$  deposition in cortical blood vessels and capillaries in AD brains by using immunogold silver staining for  $A\beta_{40}$  and  $A\beta_{42}$  on semithin LR White-embedded tissue sections.

## 2. Materials and methods

### 2.1. Study sample

The brains of demented patients with a neuropathologic diagnosis of definite or probable AD (according to the Consortium to Establish a Registry for Alzheimer's Disease [17]) were selected from the Mary S. Easton Center for Alzheimer's Disease Research Brain Bank at the University of California, Los Angeles. The neuropathologic protocols used in evaluating autopsy cases have been described elsewhere [18]. A total of 16 AD cases selected (Table 1) were grouped on the basis of CAA severity in accordance with Vonsattel criteria [19] into severe CAA (Vonsattel grade III, 7 cases: SA1 to SA7) and mild CAA (Vonsattel grade I, 9 cases: MA1 to MA9). All demented patients showed definite AD neuropathologic features, with Braak & Braak stage VI [20], except for case SA2 that had "mixed" probable AD/vascular

dementia, with Braak & Braak stage V neuropathologic findings. Non-demented controls consisted of 5 old controls (OC1 to OC5) and 4 young controls (YC1 to YC4), which at autopsy showed no significant neuropathologic changes. Included in our present study for immunogold silver staining were LR White-embedded tissue blocks from the occipital cortex. In case the occipital cortical blocks were not available for a given case, those from the frontal cortex were used (Table 1).

## 2.2. LR White embedment

Fresh brain tissue fragments of approximately 2 mm in greatest dimension were fixed at autopsy with 0.1 M Sorensen's phosphate buffer (pH 7.4) containing 2% paraformaldehyde, 0.5% glutaraldehyde, 2% sucrose, 0.15 M sodium chloride and 0.15 mg/ml calcium chloride for 2 hr and then washed with Sorensen's buffer (3 times, 30 min each). Following full dehydration with a graded ethanol series in Sorensen's buffer (50, 70, 70, 100 and 100%, 30 min each), the tissue fragments were gradually infiltrated with a mixture of medium-grade LR White resin (Ted Pella, Redding, CA, USA) and ethanol (1:2, 1:1 and 2:1) at room temperature for 1 hr each, and then with neat LR White resin at room temperature (2 times, 1 hr each), at 4°C (overnight) and at room temperature (1 hr). The tissue fragments were embedded with neat LR White resin in airtight gelatin capsules (size 00, Ted Pella) at 60°C for 24 hr to allow thermal polymerization.

## 2.3. Immunogold silver staining

LR White-embedded tissue block faces were trimmed to an approximately 2 × 3 mm size. Serial 1 micrometer-thick tissue sections were cut with a motorized ultramicrotome equipped with a diamond knife, and transferred to Fisherbrand Superfrost/Plus microscope slides (3 consecutive sections per slide). One slide from each tissue block was stained with 0.4% toluidine blue and reviewed to confirm the presence of cerebral cortical tissue, one was used for Aβ40 immunostaining, and one for Aβ42 immunostaining. Following incubation with formic acid (1:200 dilution of 90% solution in deionized water [Barnstead Nanopure Diamond, Thermo Scientific, Waltham, MA, USA]) at room temperature for 10 min, the sections were rinsed in water and in PBS. The sections were then incubated with 0.05 M glycine in PBS for 5 min to quench aldehyde groups left in tissue from fixation, rinsed in PBS, and then incubated for 10 min with blocking solution (0.5% bovine serum albumin and 0.1% cold-water-fish-skin gelatin in PBS). The sections were blotted and incubated with rabbit polyclonal anti-Aβ40 (Millipore, Temecula, CA, USA, #AB5074P) or anti-Aβ42 (Millipore, #AB5078P) primary antibody diluted 1:200 in the blocking solution (for both antibodies), at 4°C for 24 hr. After being rinsed with the blocking solution, the sections were incubated with Fab' fragments of goat anti-rabbit IgG secondary antibody conjugated with 1.4 nm-in diameter Nanogold (Nanoprobes, Yaphank, NY, USA, #2004; 1:100 dilution in 1% normal goat serum in the blocking solution) at room temperature for 3 hr. Following PBS wash and water wash, the sections were incubated with silver solution (LI Silver Enhancement Kit, Nanoprobes, #2013) at room temperature for 30 min. The sections were then rinsed in water and in PBS, and incubated with 1.25% neutrally buffered glutaraldehyde in PBS at room temperature for 3 min. After being rinsed in water, the sections were counterstained with 0.4% toluidine blue at room temperature for 1 min. Following water wash, the sections were blotted, air dried, and mounted with Cytoseal 60 (Richard-Allan Scientific, Waltham, MA, USA). Two negative reagent controls were made, one by replacing the primary antibody with the primary-antibody diluent, and the other by replacing secondary antibody with the secondary-antibody diluent.

## 2.4. Qualitative assessment of Aβ immunoreactivity

By light microscopy, immunostained sections were qualitatively evaluated independently by two neuropathologists (V. S. and H. V. V.) for the distribution patterns of Aβ40 and Aβ42

deposits in blood vessels and capillaries. On each tissue section, the presence of parenchymal A $\beta$ 40 or A $\beta$ 42 plaques served as a built-in positive tissue control. No immunogold silver staining signals were observed in any of the negative reagent controls. The differential distribution of A $\beta$ 40 and A $\beta$ 42 immunoreactive deposits in vessel walls was assessed with regard to their relative locations within the vascular wall (ie, the intima, media and adventitia), relative extents of mural involvement (ie, greater vs. less than half of the mural thickness), and longitudinal distribution patterns (ie, continuous vs. segmental, and uniformly vs. unevenly spaced). In capillary walls, the presence of A $\beta$ 40 or A $\beta$ 42 immunoreactive deposits radiating from their walls into the surrounding neuropil (“pericapillary deposits”) was recorded, as were the longitudinal distribution patterns. A $\beta$ 40 and A $\beta$ 42 plaques in cortical parenchyma were classified into diffuse plaques and dense-core plaques. The relative abundance of A $\beta$ 40 and A $\beta$ 42 plaques of diffuse or dense-core type was evaluated on the adjacent tissue sections on a scale of 1+ to 3+, with no attempt to systematically count the plaques. The relative immunostaining intensity of A $\beta$ 40 and A $\beta$ 42 plaques was also assessed. In addition, the presence of close spatial relationship between A $\beta$ -laden capillaries and A $\beta$  plaques was recorded.

### 3. Results

For each case, the demographic data, postmortem delay, fresh brain weight, cerebral cortical region examined, and presence or absence of A $\beta$ 40- and A $\beta$ 42-laden blood vessels and capillaries are shown in Table 1. The patients' ages at death ranged from 79 to 96 years (mean 86.1, standard deviation [SD] 5.3) in the severe CAA group (4 female and 3 male), from 63 to 91 years (mean 77.9, SD 11.5) in the mild CAA group (6 female and 3 male), from 67 to 86 years (mean 75.4, SD 7.8) in the old control group (1 female and 4 male), and from 13 to 43 years (mean 28.5, SD 13.2) in the young control group (2 female and 2 male).

#### 3.1. A $\beta$ deposition in blood vessel walls

In AD brains, both A $\beta$ 40 and A $\beta$ 42 deposits in vessel walls were observed in 6 of 7 severe CAA and 1 of 9 mild CAA brains. A $\beta$ 42 only-laden vessels were present in 1 of 9 mild CAA brains. A $\beta$ 40 and A $\beta$ 42 deposits were unevenly distributed along the adventitia and among the medial smooth muscle cells of leptomeningeal arteries (Figs. 1A-F) and cortical arteries and arterioles (Figs. 1G-K). In the majority of AD brains that exhibited both A $\beta$ 40 and A $\beta$ 42 deposits in vessel walls, A $\beta$ 40 immunoreactivity appeared greater than that of A $\beta$ 42 in the walls of leptomeningeal and cortical arteries on the adjacent tissue sections, with A $\beta$ 42 being preferentially located on their abluminal regions (Figs. 1E-I); the opposite immunoreactivity pattern was not observed in any of these AD brains. Nonetheless, this differential distribution of A $\beta$ 40 and A $\beta$ 42 deposits was not always obvious in the walls of arterioles on the adjacent tissue sections. Note that no A $\beta$ 40 or A $\beta$ 42 deposits were seen in any of the leptomeningeal or cortical vessels in any of the old or young non-demented controls.

#### 3.2. A $\beta$ deposition in capillary walls

In AD brains, either A $\beta$ 40 or A $\beta$ 42 deposits or both in capillary walls were observed in 6 of 7 severe CAA and 1 of 9 mild CAA brains. These A $\beta$  deposits were unevenly distributed, with a marked variation in thickness and focally abluminal excrescences (Figs. 1L-Q). No qualitative difference between A $\beta$ 40 and A $\beta$ 42 immunoreactivity was appreciated in capillary walls. In 5 of 7 severe CAA brains, a subset of A $\beta$ -laden capillaries showed pericapillary deposits of either A $\beta$ 40 or A $\beta$ 42 or both (Figs. 1R-Y, Table 1). No pericapillary A $\beta$  deposits were seen in any of the mild CAA brains. No A $\beta$ -laden capillaries were found in any of the non-demented controls.

### 3.3. A $\beta$ deposition in parenchymal plaques

In AD brains, both A $\beta$ 40 and A $\beta$ 42 plaques exhibiting diffuse or dense-core morphology were present in cortical parenchyma, A $\beta$ 42 plaques being more abundant on the adjacent tissue sections (Fig. 2A). On a scale of 1+ to 3+, the approximate extent of greater abundance of A $\beta$ 42 plaques compared to A $\beta$ 40 plaques was 1-level (ie, 2+ vs. 1+, or 3+ vs. 2+, data not shown). A $\beta$ 42 immunostaining intensity appeared in most instances greater than that of A $\beta$ 40 in parenchymal plaques on the adjacent tissue sections. Occasionally seen were A $\beta$  plaques in close proximity to A $\beta$ -laden arterioles (Fig. 2B) or capillaries (Fig. 2C). Diffuse A $\beta$ 42 plaques were observed in 1 of 5 old controls and none of the young controls. No A $\beta$ 40 plaques were seen in any of the non-demented controls.

## 4. Discussion

By using immunogold silver staining for A $\beta$ 40 and A $\beta$ 42 on semithin LR White-embedded sections of cerebral cortical tissue from AD brains, we have demonstrated high-definition patterns of A $\beta$ 42 and A $\beta$ 40 immunoreactivity in vessel walls that are consistent with deposition within the vascular basement membranes. Our observation in resin-embedded tissue, together with the findings in other studies using paraffin immunohistochemistry [10,21] and immunoelectron microscopy [16], supports a hypothesis that A $\beta$  deposits occur in cerebral perivascular drainage pathways. In vessel walls with advanced A $\beta$  deposition, a predominance of A $\beta$ 40 over A $\beta$ 42 load was apparent with A $\beta$ 42 being preferentially located on their abluminal portion, a finding in agreement with those in other studies performed on paraffin sections of sporadic AD brains [5,22].

Experiments with soluble tracers injected into the brain parenchyma of live rodents and rabbits have indicated that perivascular pathways existed for interstitial fluid drainage along the basement membranes of capillaries and vascular smooth muscle cells [9,23]. In human brains, the ultrastructure of perivascular spaces has been described [10,24]. Neuronal tauopathy in the cholinergic nucleus basalis of Meynert in advancing age [25] may contribute to deficiencies in intracortical vasodilatation, arteriolar pulsation and consequently perivascular drainage of interstitial fluid. Degenerative changes such as increased perivascular collagen deposition and thickening of the vascular basement membranes [26] may also compromise cerebral perivascular interstitial fluid drainage. These aging-related functional and structural changes of intracortical vessels [26] might promote soluble A $\beta$  to accumulate and be transformed into A $\beta$  oligomers, insoluble A $\beta$  intermediates and A $\beta$  fibrils within vascular basement membranes and parenchymal plaques [27,28].

It remains controversial as to whether deposition of A $\beta$  in capillary basement membranes occurs early on following deficiencies in A $\beta$  elimination via cerebral perivascular drainage pathways in advancing age, and whether a subset of parenchymal A $\beta$  plaques originate from A $\beta$ -laden capillaries [28]. In the brains of patients carrying the Flemish amyloid precursor protein gene mutation (A692G), an abundance of vascular and parenchymal A $\beta$  (primarily A $\beta$ 40) deposits, as well as large vasocentric dense-core A $\beta$ 40 plaques, were noted in the cerebral cortex [29]. In Tg2576 and PSAPP mouse models of AD, the majority of dense-core A $\beta$  plaques were shown to be centered on capillaries [15]. In a study by Miyakawa et al [30] on parahippocampal tissue from 10 AD and 10 control brains, close spatial association of almost all dense-core A $\beta$ 40 plaques with capillaries was shown on double immunofluorescent staining for A $\beta$  and collagen IV. On electron microscopy, they found that some degenerated capillaries containing amyloid fibrils in their walls were localized in close proximity to dense-core amyloid plaques [30]. An immunoelectron microscopic study of AD brains by Yamaguchi et al [16] has suggested that the capillary basement membrane was one of the predilection sites for early A $\beta$  deposition. Also in this report, pericapillary A $\beta$  deposits were shown extending from the outer basement membranes of A $\beta$ -laden capillaries [16]. Preston et al [10] reported



that diffuse A $\beta$  plaques were seen surrounding capillaries, while dense-core A $\beta$  plaques were not associated with blood vessels. In the same study, confocal microscopy revealed pericapillary deposits of amyloid on thioflavin-S staining in continuity with amyloid in the capillary walls [10]. On the other hand, by using double immunostaining for A $\beta$  and collagen IV on the entorhinal cortex of two AD patients, Kawai et al [31] suggested that spatial association between capillaries and both diffuse and dense-core A $\beta$  plaques occurred by chance, depending on the size of A $\beta$  plaques. A $\beta$ -laden capillaries originally associated with diffuse plaques may progressively be loaded with A $\beta$  and undergo degeneration, as diffuse plaques evolve into dense-core plaques [30]. Therefore, there would be a higher probability for identifying A $\beta$ -laden capillaries within diffuse A $\beta$  plaques than in dense-core A $\beta$  plaques on immunohistologic sections studied either at the light or electron microscopic level.

Pericapillary A $\beta$  deposits found in our present study may represent a histopathologic intermediate in the course of development from capillary A $\beta$  deposits to diffuse A $\beta$  plaques. It has been shown that the A $\beta$ 40/42 ratio of A $\beta$ -laden capillaries is lower than that of affected arteries but comparable to that found in A $\beta$  plaques [32]. As a result of impaired drainage along cerebral perivascular pathways in old age, the less soluble A $\beta$ 42 is unable to drain as far along the clearance channels as the more soluble A $\beta$ 40. The A $\beta$ 42 thus accumulates, becomes insoluble, and predominantly deposits in brain parenchyma in the form of plaques. In contrast, the A $\beta$ 40 deposits preferentially in the basement membranes of cortico-leptomeningeal vessels to form CAA [28]. This working hypothesis would explain the consistent findings in sporadic AD brains of a predominance of A $\beta$ 40 over A $\beta$ 42 load in vessel walls and the opposite pattern in parenchymal plaques [2,5,22], which were also shown in our present qualitative analysis.

Regarding technical aspects, the immunogold silver staining of semithin resin-embedded tissue sections was useful as demonstrated in our present study, particularly in evaluating the cerebral vasculature, because the localization of protein markers of interest can be correlated more precisely with high-definition histology compared to paraffin immunohistochemistry. Immunoreactive substances (A $\beta$ 40 and A $\beta$ 42) can be seen in relation to vascular medial smooth muscle cells or capillary endothelium. Use of hydrophilic acrylic resins that can relatively preserve the antigenicity of tissue (eg, LR White resin) complemented by careful optimization of the fixation, dehydration, embedment and staining protocols is critical for high-definition immunohistologic studies on semithin tissue sections. We obtained consistently satisfactory results of post-embedding immunogold silver staining following full tissue dehydration in absolute ethanol and embedment in medium-grade LR White resin by thermal polymerization. Nonetheless, other investigators have proposed different modified methods shown to improve the antigenicity and ultrastructure of LR White-embedded tissue. For instance, Yamaguchi et al [16] pursued partial dehydration of tissue in 70% ethanol before embedment in hard-grade LR White resin by chemical catalytic polymerization at 0°C, while Sakai et al [33] treated tissue with 1% tannic acid prior to partial dehydration in 70% ethanol containing 2% phosphotungstic acid, followed by embedment in LR White resin by thermal polymerization. In our immunogold silver staining protocols for A $\beta$ 40 and A $\beta$ 42 on semithin LR White-embedded tissue sections we successfully applied 90% formic acid pretreatment at 1:200 dilution for 10 min to unmask antigens, in contrast to standard pretreatment with neat formic acid solution for paraffin A $\beta$  immunohistochemistry [34]. For other protein markers, different antigen retrieval techniques may be needed on resin-embedded tissue sections.

The relatively small field size of semithin resin-embedded tissue sections that is subject to sampling errors may be considered a drawback to the study of human brains; nonetheless, examination of multiple serial sections on the same slide is feasible. When this method is applied to immunohistologic analysis of rodent brains in animal models of neurological diseases [15], sampling errors are of lesser concern. The presence of A $\beta$ -laden blood vessels in 6 of 7 severe CAA brains but in only 2 of 9 mild CAA brains in our present study can

probably be explained by lower detection sensitivity of this method compared to much larger and thicker tissue sections used in paraffin immunohistochemistry. By using 14 micrometer-thick paraffin sections of AD brains, Alonzo et al [5] found no significant difference in the proportion of A $\beta$ 40- or A $\beta$ 42-immunostained vessels between mild and severe CAA (classified according to the Vonsattel system, which qualitatively evaluates the degree of CAA involvement of individual vessels [19]). We found A $\beta$ -laden capillaries in 6 of 7 severe CAA brains but in only 1 of 9 mild CAA brains. This observation differed somewhat from a report by Thai et al [12] showing that the degree of CAA severity did not predict the presence of capillary CAA. Nonetheless, CAA with capillary involvement may be pathogenetically distinct from that lacking capillary involvement, as only the former was found to correlate with the apolipoprotein E  $\epsilon$ 4 allele [12].

In summary, our study demonstrated high-definition characteristics of A $\beta$ 40 and A $\beta$ 42 deposition in the cerebral vasculature. The patterns of A $\beta$ 42 and A $\beta$ 40 immunoreactivity in blood vessel walls suggest that A $\beta$  deposits occur in the vascular basement membranes along cerebral perivascular drainage pathways, extending from cortical capillaries to leptomeningeal arteries. The presence of pericapillary A $\beta$  deposits supports the notion that a subset of A $\beta$  plaques originate from A $\beta$ -laden capillaries. As significant heterogeneity exists among individuals with AD diagnosis [35], this proposed mechanistic cascade of cerebral microvascular and parenchymal deposition of A $\beta$  may be applied only to those individuals whose brains exhibit preferential capillary involvement such as type-1 CAA described by Thai et al [12]. Acknowledging that sporadic AD is pathogenetically heterogeneous, we propose that the validity of classifying CAA based on the presence or absence of capillary involvement be taken into account when studying the pathogenesis of this complex disease.

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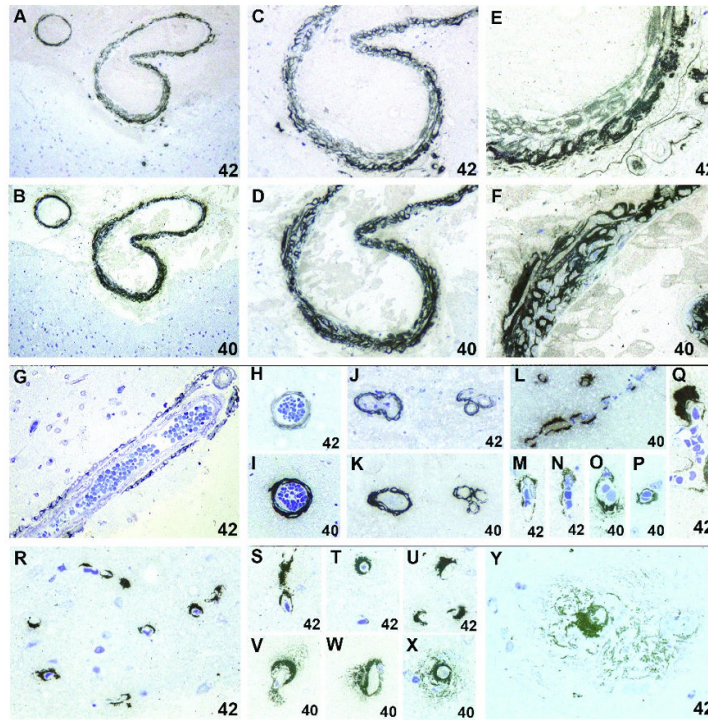
## List of nonstandard abbreviations

### Used in the manuscript

<b>A<math>\beta</math></b>	$\beta$ -amyloid
<b>AD</b>	Alzheimer's disease
<b>CAA</b>	cerebral A $\beta$ angiopathy
<b>SA</b>	brains of AD patients with severe CAA
<b>MA</b>	brains of AD patients with mild CAA
<b>OC</b>	brains of non-demented old controls
<b>YC</b>	brains of non-demented young controls

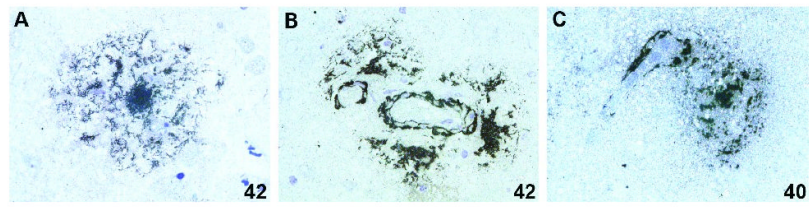
### Used exclusively in the table

<b>F</b>	female
<b>M</b>	male
<b>RO</b>	right occipital
<b>LO</b>	left occipital
<b>RF</b>	right frontal
<b>“+”</b>	presence
<b>“–”</b>	absence
<b>PD</b>	pericapillary deposits



**Fig. 1.**

Leptomeningeal arteries show A $\beta$ 42 (A, C and E) and A $\beta$ 40 (B, D and F) deposits in the adventitia and among the vascular smooth muscle cells of the media. While A $\beta$ 40 deposits are distributed through most of the vessel wall (F), those of A $\beta$ 42 are preferentially located on their abluminal regions (E). In cortical vessels, A $\beta$ 42 (G, H and J) and A $\beta$ 40 (I and K) deposits are distributed in a pattern similar to that seen in leptomeningeal vessels. Note that pairs of A $\beta$ 42- and A $\beta$ 40-immunostained vessels shown in (A-F) are from the adjacent tissue sections, as are those in (H and I) and (J and K). In capillary walls, A $\beta$ 40 (L, O and P) and A $\beta$ 42 (M, N and Q) deposits are unevenly distributed, with a marked variation in thickness and focally abluminal excrescences. In a subset of A $\beta$ -laden capillaries, there are A $\beta$ 42 (R-U and Y) and A $\beta$ 40 (V-X) deposits radiating from their walls into the surrounding neuropil (“pericapillary deposits”). Original magnification  $\times 200$  (A and B),  $\times 400$  (C, D and G-L), and  $\times 1000$  (E, F and M-Y). Cases SA7 (A-F), SA5 (G-P, X and Y), and SA4 (Q-W).



**Fig. 2.**

A dense-core Aβ42 plaque (A, case SA6). Aβ42 plaques are in close proximity to an Aβ42-laden arteriole (B, case MA8). An Aβ40 plaque is in close proximity to an Aβ40-laden capillary (C, case SA5). Original magnification ×1000 (A-C).

**Table 1**  
Summary of demographic data and immunohistologic findings in autopsy specimens

Case	Age (yr)-Sex	Postmortem delay (hr)	Brain weight (g)	Cerebral cortex	A $\beta$ 40 artery / arteriole	A $\beta$ 42 artery / arteriole	A $\beta$ 40 capillary	A $\beta$ 42 capillary
SA1	86-F	19	970	RO	+	+	+	-
SA2	85-M	4	1250	RO	-	-	-	-
SA3	87-F	14	925	LO	+	+	+	+
SA4	83-F	24	1320	RO	+	+	+	+
SA5	88-M	14	1120	RF	+	+	+	+
SA6	79-M	21	1100	LO	+	+	+	+
SA7	96-F	12	920	RO	+	+	-	+
MA1	88-F	6	No record	LO	-	-	-	-
MA2	74-M	12	1160	RO	-	-	-	-
MA3	90-M	13	1110	RO	-	-	-	-
MA4	65-F	9	1160	RF	-	-	-	-
MA5	72-F	16	950	RO	-	-	-	-
MA6	91-F	4	1120	RO	+	+	-	-
MA7	63-F	18	950	LO	-	-	-	-
MA8	69-F	16	910	RO	-	+	-	+
MA9	89-M	15	1165	RO	-	-	-	-
OC1	72-F	16	1150	RO	-	-	-	-
OC2	86-M	28	1170	RO	-	-	-	-
OC3	67-M	22	1670	LO	-	-	-	-
OC4	81-M	24	1470	RO	-	-	-	-
OC5	71-M	26	1270	RO	-	-	-	-
YC1	43-F	30	No record	RO	-	-	-	-
YC2	35-F	15	1560	LO	-	-	-	-
YC3	23-M	21	1570	LO	-	-	-	-
YC4	13-M	22	1430	LO	-	-	-	-

Abbreviations: A $\beta$ ,  $\beta$ -amyloid; SA1-SA7, brains of Alzheimer's disease patients with severe cerebral A $\beta$  angiopathy (Vonsattel grade III); MA1-MA9, brains of Alzheimer's disease patients with mild cerebral A $\beta$  angiopathy (Vonsattel grade I); OC1-OC5, brains of non-demented old controls; YC1-YC4, brains of non-demented young controls; F, female; M, male; RO, right occipital; LO, left occipital; RF, right frontal; "+", presence; "-", absence; PD, pericapillary deposits (ie, A $\beta$  deposits radiating from A $\beta$ -laden capillaries into the surrounding neuropil).