American Journal of Pathology, Vol. 142, No. 5, May 1993

Distribution of β/A4 Protein and Amyloid Precursor Protein in Hereditary Cerebral Hemorrhage with Amyloidosis–Dutch Type and Alzheimer's Disease

Annemieke J.M. Rozemuller,*,† Raymund A.C. Roos,‡ Gerard T.A.M. Bots,‡ Wouter Kamphorst,* Piet Eikelenboom,† and William E. Van Nostrand§

From the Department of Pathology and the Department of Psychiatry,† Free University Hospital, Amsterdam, The Netherlands; the Departments of Neurology and Neuropathology,‡ University Hospital, Leiden, The Netherlands; and the Department of Microbiology and Molecular Genetics,§ University of California, Irvine, California*

Brain amyloidosis with abundant β/A4 protein deposition in plaques and cortical and meningeal vessels is found in Alzheimer's disease (AD) and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). In contrast to AD, no neuritic pathology or classical congophilic plaques are found in HCHWA-D. Unlike most AD cases, the congophilic angiopathy in HCHWA-D is very severe. It is still unknown whether β/A4 deposits in plaques and vessels have the same origin. In this study, we have used frozen cortical tissue of HCHWA-D and AD patients to investigate the β/A4 amyloid protein and the amyloid precursor protein (APP) in different types of plaques and congophilic angiopathy. Immunohistochemical staining was conducted using antibodies against synthetic β/A4 proteins and antibodies against APP including MAbP2-1, a monoclonal antibody against purified protease nexin-2, which is the secreted form of APP. In contrast to immunohistochemical studies on formalin-fixed, paraffin-embedded tissue, frozen tissue of HCHWA-D patients revealed a very high number of β/A4 plaques resembling AD. All plaques were of the diffuse type. Doublestaining with MAbP2-1 and β/A4 antisera revealed 1) the presence of APP immunoreactivity in classical plaques and transitional forms; 2) the absence of APP immunoreactivity in diffuse plaques in HCHWA-D and AD; and 3) pronounced APP immunoreactivity in congophilic vessels in HCHWA-D in contrast to weak APP staining in congophilic vessels in AD. Together these findings suggest that a) the presence of APP in plaques is related to neuritic changes; b) different processes occur in amyloid formation in plaques and vessels; and c) differences exist between the process of amyloid formation in HCHWA-D and AD. (Am J Pathol 1993, 142: 1449–1457)

Amyloidosis of the central nervous system and formation of paired helical filaments[1] (PHF) are the most characteristic pathological features of patients with Alzheimer's disease (AD)[2] and aged patients with Down Syndrome.[3] The amyloid protein in these disorders is the 4-kd β/A4 protein,[4–7] which is derived from a larger membrane-spanning molecule,[8,9] the amyloid precursor protein (APP).[10–12] The β/A4 protein contains half of the transmembrane region and the first 28 aminoacids of the extracellular domain.[6,7] The normal processing of APP, involved with the release of the extracellular domain known as protease nexin-2 (PN-2),[13–15] is the result of proteolytic cleavage through the β/A4 domain of APP.[16] Releasing of intact β/A4 occurs through aberrant proteolysis of APP caused by unknown brain specific processes.[16,17]

β/A4 deposits are found as senile plaques dispersed in the neuropil of the cortex, hippocampus, and subcortical nuclei. Several types of β/A4 plaques have been described, including the classical amyloid

Supported by the Dutch Praevention fund grant 28-1945 (J.M.R.) and the National Institutes of Health grant AG 00538 (W.E.V.N.).

Accepted for publication October 22, 1992.

Address reprint requests to Dr. Annemieke J.M. Rozemuller, Institute of Pathology, Free University Hospital, Postbox 7057, 1007 MB Amsterdam, The Netherlands.

plaque surrounded by dystrophic neurites and glial cells[18] and the amorphous or diffuse, noncongophilic plaque,[19–28] which seems to be an early stage in amyloid plaque formation.[19,25,26] In addition to amyloid in plaques, amyloid is found in blood vessel walls in some AD patients. Vascular amyloid is localized in the media and adventitia of cortical and meningeal small- and medium-sized arterioles and capillaries.[29–31] This phenomenon is called congophilic angiopathy or cerebral amyloid angiopathy. Plaques and vascular amyloid can also be found in aged nondemented controls, though less abundantly.

Messenger RNA for APP, which is encoded by a gene on chromosome 21,[8,10–12] is expressed in many cell types within and outside the brain.[10,32,33] Recently, mutations in the β/A4 region of the APP protein have been described in a few families with hereditary AD[34–37] but not in other AD families or the sporadic AD cases. In a rare form of brain amyloidosis, called hereditary cerebral hemorrhage with amyloidosis-dutch type (HCHWA-D), severe congophilic angiopathy is found in cortical and meningeal vessels at an early age[38–41] due to a mutation at position 22 of the β/A4 protein.[42–44] In patients with HCHWA-D, a variable number of diffuse, noncongophilic plaques have been described,[38,40] but no neuritic pathology.[40,41,45] Because there is an absence of classical, congophilic plaques in this tissue,[38,40,41] HCHWA-D patients provide a unique model for the study of diffuse plaque formation.

The origin of the β/A4 protein remains unclear. A vascular, glial, neuronal, or extracerebral source has been suggested. It is also unknown whether plaque amyloid and vascular amyloid are induced by the same proteolytic processes. To understand further amyloid formation in plaques and vascular amyloid, we have investigated immunohistochemically the distribution of APP and β/A4 proteins in patients with HCHWA-D and compared the findings with those in AD patients.

Materials and Methods

Brain Tissue

Brain tissue was obtained at autopsy from four HCHWA-D patients, five AD patients, and three controls (see Table 1). AD and control brain tissue was obtained from the Netherlands Brain Bank, Amsterdam (Coordinator Dr. R. Ravid). Diagnosis was confirmed using routine histochemical staining techniques (hematoxylin and eosin staining, Bodian silver, modified Bielschowsky or periodic methenamine silver staining, Congo red staining) performed on formalin-fixed, paraffin-embedded tissue (fixation in 10% formalin for 4 to 5 weeks). Small pieces of parietal, temporal, and/or occipital cortex were frozen in liquid nitrogen for immunohistochemical studies. Immunohistochemical staining was also performed on routine fixed, paraffin-embedded cortical tissue. In addition to numerous plaques, AD patients were selected for the presence of congophilic angiopathy.

Table 1. *Age, Sex, Duration of the Disease, and Clinical Diagnosis of Patients Used in This Study*

Patient No.	Age	Sex	Duration (years)	Diagnosis	Weight (g)
1	81	F	>2	SDAT	990
2	64	M	6	AD	1355
3	40	M	5	AD	1370
4	64	M	7	AD	1210
5	48	M	8	AD	1435
1	47	F	2	HCHWA-D	1250
2	42	M	1 month	HCHWA-D	1500
3	50	M	5	HCHWA-D	1360
4	51	M	3	HCHWA-D	1400
1	73	M	-	Nondemented	1410
2	51	F	-	Nondemented	1190
3	71	F	-	Nondemented	1240

Antibodies

Mouse monoclonal antibodies (MAbs) and antisera in this study included MAbP2-1,[13–15] which recognizes an N-terminal epitope on all major isoforms of APP, and rabbit antiserum APP-45 (gift Dr. K. Beyreuther) against total APP-695, MAb22C11 (Boehringer), rabbit anti-A4-antisera 63122[7] (gift Dr. C.L. Masters and Dr. K. Beyreuther), and SP28[46] (courtesy of Dr. B. Frangione) against respectively 1-42 and 1-28 synthetic β/A4 proteins, NFT200[47] (Innogenetics) against PHF and dystrophic neurites, and a MAb against glial fibrillary acidic protein (Monosan).

Immunohistochemical Staining

Staining with MAbP2-1, APP-45, MAb22C11, anti-A4, SP28, anti-GFAP, and anti-PHF antibodies was performed on frozen tissue. Then 8-μ-thick serial cryostat sections were mounted on poly-L-lysine-coated glass slides, air-dried, and fixed in acetone or a buffer with 4% paraformaldehyde for ten minutes. A4-antisera, APP-45, and MAb22C11 were also used for immunohistochemistry on formalin-fixed tissue. Eight-μ-thick paraffin sections were then mounted on poly-L-lysine- (or chrome-alum

gelatine–) coated glass slides, dehydrated in ethanol, and preincubated in 0.3% H_2O_2 to block endogenous peroxidase. Paraffin sections were pretreated with 85% formic acid[48] before β/A4 staining.

All antibodies were appropriately diluted in phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumin. Immunolabeling for MAbP2-1 was demonstrated with the indirect technique, using peroxidase labeled rabbit anti-mouse antisera (DAKO, Denmark) as a second antibody. Immunolabeling for rabbit antisera (A4, APP-45, SP28) was demonstrated with the peroxidase-antiperoxidase technique.[49] These sections were preincubated with normal swine serum. Peroxidase activity was revealed by the DAB method. (5 mg 3,3-diaminobenzidine [DAB] in 10 ml phosphate-buffered saline, pH 7.4, containing 0.02% H_2O_2 for 3 to 5 minutes). After immunoperoxidase staining, sections were counterstained with Congo red[50] (if not pretreated with formic acid) to visualize the amyloid, then dehydrated, and mounted. Specificity of the polyclonal antiserum APP-45 was confirmed by absorption with purified PN-2/APP. Antiserum APP-45 was incubated overnight at 4 C with several dilutions of PN-2/APP ranging from 1 mmol to 100 mmol and centrifugated at 10,000*g*. The supernatant was used as primary antibody.

Double Staining

Double staining was performed on frozen tissue by incubating simultaneously with anti-β/A4 rabbit polyclonal antibodies and MAbP2-1, followed in the second step by an incubation with alkaline-phosphatase-labeled goat anti-rabbit (Tago, Burlingame, CA) and biotinylated horse anti-mouse (Vector Laboratories, Burlingame, CA). In the third step, sections were incubated with peroxidase-labeled avidin-biotin-complex (Vector Elite kit). Peroxidase activity was visualized using the DAB method. Alkaline phosphatase was revealed using naphthol AS-MX phosphate as substrate and Fast Blue BB as coupling agent. Secondary antisera and reagents were tested for lack of cross-reactivity and nonspecific staining.

Results

Our immunohistochemical findings are summarized in Table 2. In cryostat brain sections of AD, HCHWA-D, and controls, neurons (soma and part of the axon) were immunoreactive with APP-antibodies (Figure 1). Tangle-bearing cells identified by Congo red staining did not show APP-labeling of the intracellular tangles. Glial cells identified by MAbs against glial fibrillary acidic protein, remained unstained for β/A4 or APP. In all cases, a faint staining for APP could be found in cortical and subcortical microvessels.

Plaques

Immunostaining with antibodies against synthetic β/A4, (i.e., anti-A4 [63122] and SP28) showed identical results on adjacent sections. Immunoreactivity was found in numerous plaques in HCHWA-D patients and AD patients. In HCHWA-D patients, the density of β/A4 plaques was much higher in frozen sections than in paraffin sections immunostained for β/A4 or stained using periodic methenamine silver method (40 to 70 plaques/mm^2 and 15 to 40 plaques/mm^2, respectively). Counterstaining with Congo red showed no birefringence of amyloid in HCHWA-D, indicating that these plaques are of the diffuse type (Figure 2). Comparing β/A4 and APP staining in HCHWA-D cases on adjacent sections revealed that diffuse, β/A4-positive plaques did not show reactivity for APP-45 antiserum and MAbP2-1. However, very rare APP-positive deposits were found in two out of four patients. Similarly, brain sections of HCHWA-D patients doublestained with MAbP2-1 and A4 antisera revealed numerous A4-positive but APP-negative plaques (Figure 1). In AD brains, the density of β/A4-immunostained plaques

Table 2. *Immunohistochemical Staining for Antibodies against β/A4 and APP in Diffuse and Classical Plaques, Vascular Amyloid, and Neuropil Threads*

Stain	Diffuse plaques HCHWA-D	AD	Classical plaques HCHWAD-D	AD	Vascular amyloid HCHWA-D	AD	Neuropil threads
Congo red	–	–	np	+	+	+	–
β/A4	+	+	np	+	+	+	–
SP28	+	+	np	+	+	+	–
MAbP2-1	–	–/±	np	+	+	–	–
APP-45	–	–/±	np	+	+	–	–
MAb22C11	–	–/±	np	+	+	–	–

–/±, a few noncongophilic plaques are stained; np = not present.

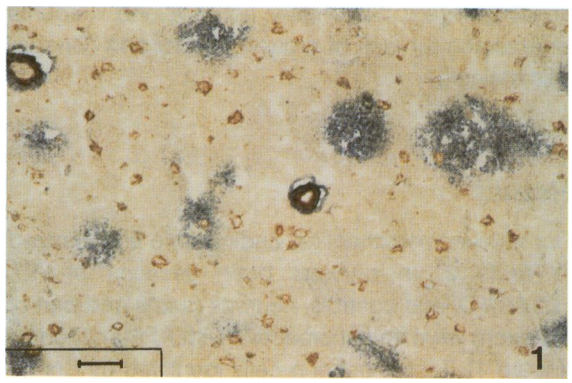
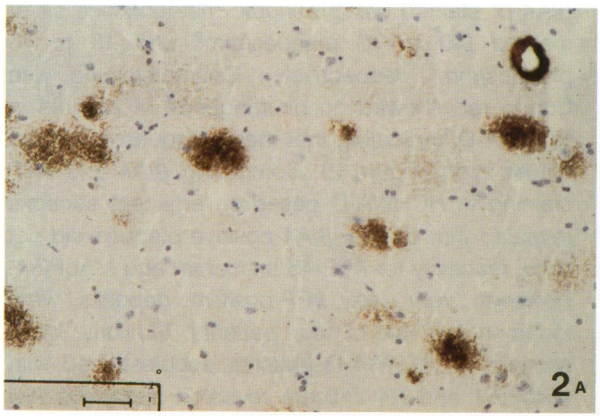


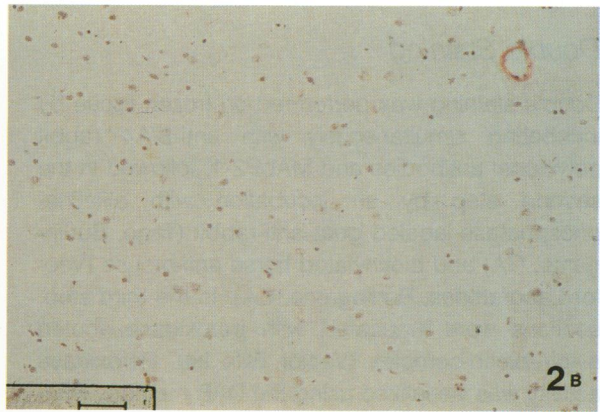
Figure 1. *Doublestaining for APP (MAbP2-1, brown) and β/A4 (rabbit 63122, blue) on frozen section of a patient with HCHWA-D. Note neuronal staining for APP but absence of APP in diffuse β/A4 plaques. Congophilic vessels are double stained for β/A4 and APP. (Scale bar = 40 μ).*

in frozen tissue was 40 to 80 plaques/mm^2. However, the density of plaques using modified Bielschowsky staining on paraffin sections of adjacent tissue varied between 25 and 70 plaques/mm^2. Counterstaining with Congo red showed that the diffuse, noncongophilic plaques outnumbered the amyloid plaques. Diffuse plaques were found in all cortical layers. Comparison of adjacent sections stained respectively for β/A4 and APP showed that in AD patients 5 to 20% of the β/A4-positive plaques showed APP immunoreactivity either in the corona of classical plaques (Figure 3) or as more diffuse irregular plaque staining. Paraformaldehyde fixation did not reveal more APP-positive plaques than acetone fixation. Doublestaining with β/A4-antisera and MAbP2-1 did not show APP-positive neurites outside the β/A4-positive plaques (Figure 4). Although APP-positive plaques were frequently congophilic, some did not reveal birefringence. Congophilic plaques but not diffuse plaques showed a corona of dystrophic neurites stained with antibodies against PHF. In control cases, some non-congophilic, β/A4-positive plaques were found (0 to 12 plaques/mm^2 in frozen sections). APP-positive deposits were rarely seen. No staining was found in these cases using antibodies against PHF.

Congophilic Angiopathy

Immunohistochemical staining for β/A4 showed reactivity of tunica media and adventitia in many leptomeningeal and cortical microvessels of HCHWA-D patients and less frequently in the microvessels of AD patients. Congo red staining showed less extensive staining of the vessel wall than immunohistochemical demonstration of the β/A4 protein. No





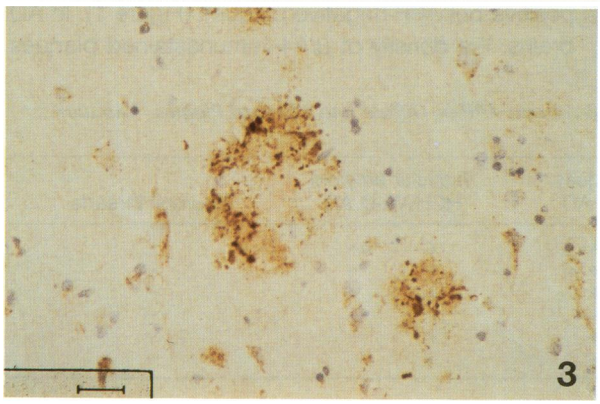


Figure 2. **A:** *Immunohistochemical staining for β/A4 protein on frozen cortical tissue of a patient with HCHWA-D.* **B:** *Adjacent section stained with Congo red. Note the discrepancy between Congo red staining and β/A4 staining. (Scale bar = 80 μ).*
Figure 3. *Immunoreactivity for APP (MAbP2-1) in the corona of a congophilic plaque on frozen tissue of a patient with AD. (Scale bar = 20 μ).*

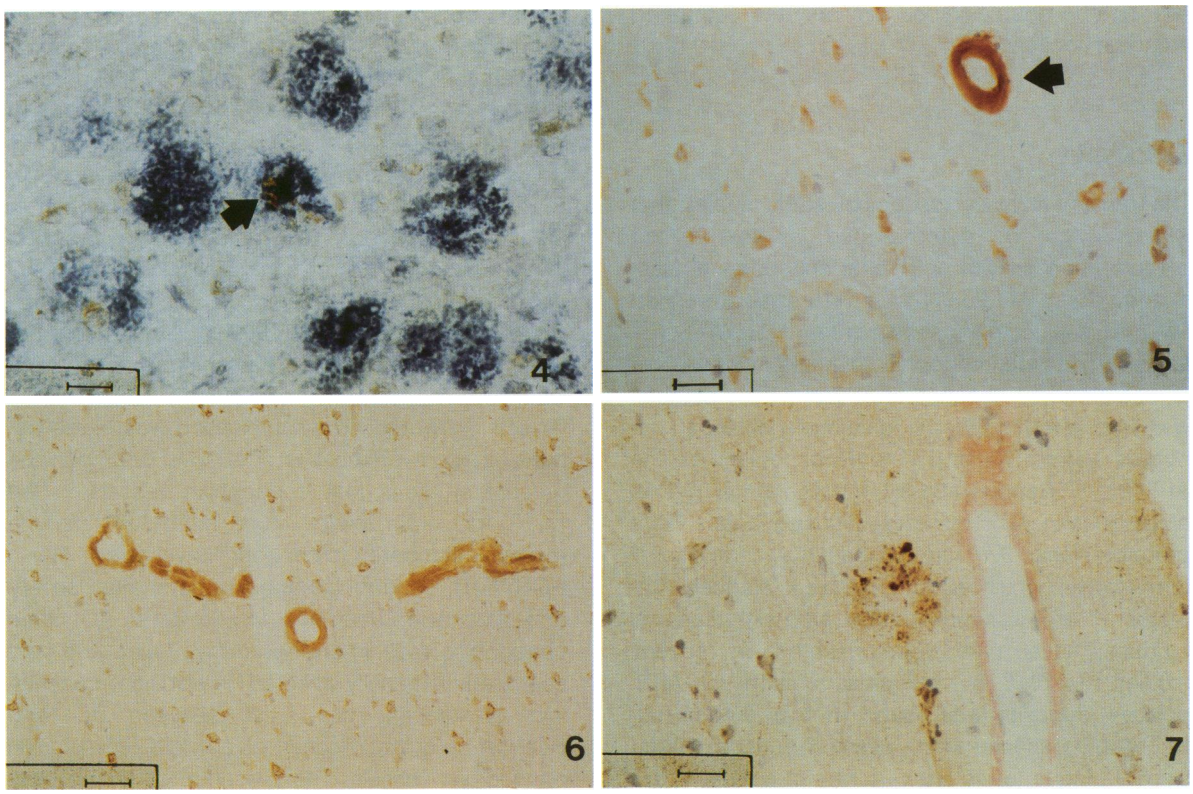


Figure 4. *Doublestaining for APP (MAbP2-1, brown) and β/A4 (rabbit 63122, blue) on frozen tissue of a patient with AD. APP-positive structures are associated with some (arrow) but not all β/A4 plaques. (Scale bar = 40 μ).*
Figure 5. *Staining for APP (MAbP2-1) on frozen tissue of a HCHWA-D patient. Note the difference of immunoreactivity of the amyloidotic vessel (arrow) and the normal vessel. (Scale bar = 20 μ).*
Figure 6. *Immunoreactivity for APP (MAbP2-1) in neurons and in congophilic vessels in a frozen section of a patient with HCHWA-D. (Scale bar = 40 μ).*
Figure 7. *Immunohistochemical staining for APP (MAbP2-1) on a frozen section of an AD patient counterstained with Congo red. Note presence of APP in the corona of a plaque but absence of APP in the adjacent congophilic vessel. (Scale bar = 20 μ).*

relationship was found between the β/A4 plaques and the congophilic vessels. Occasionally, perivascular β/A4 staining was found around congophilic vessels outside the adventitia extending into the neuropil (dyshoric angiopathy). This phenomenon was seen in some AD cases and HCHWA-D cases. In paraffin sections, staining for β/A4 of congophilic vessels was much stronger than plaque staining. This difference, however, was not seen in frozen tissue.

A combination of immunoperoxidase labeling for APP and Congo red staining in frozen sections showed that tunica media and adventitia of most congophilic vessels in HCHWA-D were more intensely stained for the APP protein (Figures 5 and 6). In contrast, amyloidotic vessels in AD patients that were counterstained with Congo red did not show this pronounced APP immunoreactivity (Figure 7). However, in the absence of Congo red counterstaining, weak immunoperoxidase staining for APP could be found in microvessels in AD patients and in controls (data not presented). Furthermore, doublestaining with MAbP2-1 and β/A4 antisera showed doublestained vessels in HCHWA-D (Figure 1) but not in AD. The vessel staining with the polyclonal antiserum was greatly reduced by prior immunoabsorption with PN-2/APP. In addition, staining of parallel sections of HCHWA-D patients with nonimmune rabbit polyclonal antiserum did not show this vessel staining.

Dystrophic Neurites

Staining with NFT200 against PHF visualized numerous tangles and dystrophic neurites (associated with plaques or isolated in the neuropil) in AD but not in HCHWA-D and controls. Dystrophic neurites were especially seen around classical plaques with a congophilic core. These plaques were also associated with APP-positive neurites. In contrast, isolated neuropil threads identified as isolated NFT200-positive threads not associated with plaques were unlabeled with antibodies against

APP both in doublestained sections and compared adjacent sections.

Discussion

The present light-microscopical study shows that APP can not be demonstrated in most diffuse plaques, neither with polyclonal antibodies against total synthetic APP, nor with a MAb against purified PN-2. This was clearly shown in four HCHWA-D patients who lacked congophilic plaques and had only diffuse plaques. Absence of APP in diffuse plaques was also noted in other studies using different antibodies against N- and C-terminal regions of the APP.[45,51–53] Reports about the presence of APP in all plaques in AD[54] including diffuse plaques[55] are probably due to APP immunoreactivity in transitional forms between diffuse and classical plaques. Detection of APP in neurites associated with classical plaques have been demonstrated in several studies.[45,51–54,56] A very small number of APP-positive deposits was found in two out of four HCHWA-D patients, which could suggest that transitional plaque forms are very rare or absent in this disease. On the other hand, a small number of APP deposits without amyloid proteins have also been described in normal controls.[56]

A recent study of AD patients by Tagliavini et al has demonstrated APP immunoreactivity in diffuse plaques with a MAb against the N-terminal region but not with an antiserum against the C-terminal segment.[57] However, the MAbs in our study that are also directed against an N-terminal region of APP[13] did not label the diffuse plaques. Importantly, the study of Tagliavini et al employed formalin fixation and paraffin embedding, whereas we used frozen tissue. It is known that formalin fixation and paraffin embedding can modify the results, especially concerning the presence of serum proteins in plaques.[58] In addition, former studies of HCHWA-D brains performed on paraffin sections[40,45,57] have reported the presence of few plaques in this disorder. In the present study, however, we have found a large number of diffuse plaques that were APP-negative. Comparative β/A4 staining with the same immunohistochemical techniques and antibodies showed that staining on frozen tissue revealed more diffuse plaques/mm^2 in frozen sections than in paraffin sections. Moreover, plaques were stained more intensely in frozen tissue. Similarly, in AD brains, diffuse plaques could be visualised better in frozen tissue. Double labeling for β/A4 and APP in frozen tissue did not show APP-positive cells or neuropil threads outside the β/A4 deposits, with the exception of the neurons. Together, the present findings suggest that previous reports about of APP-positive neurites outside the plaques[59] can be the result of less optimal staining of plaques.

Conflicting reports exist about the presence of APP in congophilic vessels. With the exception of the study of Ko et al,[60] most studies have reported the absence of APP in congophilic vessels in AD using a variety of antibodies.[51–53] A study of Tagliavini et al[45] on cortices of patients with AD and patients with HCHWA-D demonstrated the co-localization of APP and amyloid fibrils in congophilic vessels. However, no discrimination was made between these two diseases. The novel finding in the present study is that congophilic vessels in the HCHWA-D brain exhibit much more APP immune reactivity compared to congophilic vessels in AD. In HCHWA-D, congophilic angiopathy is often severe and starts at an early age, in contrast to AD where congophilic vessels are not always found and vessels are less heavily affected. In addition, quantitative differences may exist between AD and HCHWA-D in the process of amyloid formation in congophilic vessels. The presence of APP in vessels may be a function of the time course of amyloid deposition.

Previous studies have suggested that plaque amyloid derives from the vascular system.[45,61] In the present study, no relationship was found between plaques and congophilic vessels in HCHWA-D nor AD consistent with other reports.[55,62] Several differences between vascular and plaque amyloid have been described.[7,63–65] It has been suggested that diverse processing of the APP molecule occurs in vessel walls and brain parenchyma due to tissue specific enzymes.[63] In a recent ultrastructural study,[66] APP has been found in smooth muscle cells in leptomeningeal vessels, suggesting that smooth muscle cells could be the source of APP in congophilic angiopathy. On the other hand, a neuronal origin of plaque amyloid has been suggested in recent ultrastructural studies.[67,68] Differences between plaque and vascular amyloid are supported by our findings that in HCHWA-D congophilic vessels are immunoreactive for APP, whereas diffuse plaques are unstained.

Reactive neurites, identified by tau antibodies or silver staining, are found in classical plaques and transitional forms but not in diffuse plaques.[19,53] In our opinion, APP-positive structures in some of the plaques are reactive neurites in a later stage of plaque formation. β/A4 deposition is a necessary but not the sole basis for classical plaque forma-

tion.[69,70] In this regard, HCHWA-D can be used as a model for the study of different steps in plaque formation. As there are neither neuritic nor glial changes around the abundant diffuse plaques in HCHWA-D, our findings do not support the hypothesis of Kowall et al[71] that β/A4 itself should produce neurotoxic changes in the neuropil. Perhaps other factors and/or conditions are present in AD, but lacking in HCHWA-D, that are necessary to promote β/A4 neurotoxicity.

In conclusion, we report the absence of APP immunoreactive structures in diffuse plaques both in AD and in HCHWA-D. This suggests that diffuse plaques in HCHWA-D can be used as a model to study very early stages in amyloid plaque formation without neuritic changes. Furthermore, we report a striking difference between HCHWA-D and AD concerning the co-localization of APP in congophilic vessels, suggesting that different processes occur in these two disorders.

Acknowledgments

We thank Drs. C.L. Masters, K. Beyreuther, and B. Frangione for the gift of β/A4 antisera. Brain tissue was obtained from the Netherlands Brain Bank, Amsterdam (Coordinator Dr. R. Ravid).

References

1. Kidd M: Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 1963, 197:192–193
2. Alzheimer A: Ueber eigenartige Krankheitsfälle des späteren Alters. Z f d g Neur u Psych 1911, 4:356–385
3. Jervis GA: Early senile dementia in mongoloid idiocy. Am J Psychiatry 1949, 105:102
4. Glenner GG, Wong CW: Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 1984, 120:885–890
5. Glenner GG, Wong CW: Alzheimer's disease and Down's syndrome: sharing a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun 1984, 122:1131–1135
6. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K: Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J 1985, 4:2757–2763
7. Masters CL, Simms G, Weinmann NA, Multhaup G, McDonald BL, Beyreuther K: Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. Proc Natl Acad Sci USA 1985, 82:4245–4249
8. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grezeschik KH, Multhaup G, Beyreuther K, Muller-Hill B: The precursor of Alzheimer disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987, 331:530–532
9. Dyrks T, Weidemann A, Multhaup G, Salbaum JM, Lemaire HG, Kang J, Muller-Hill B, Masters CL, Beyreuther K: Identification, transmembrane orientation and biogenesis of the amyloid A4 precursor of Alzheimer's disease. EMBO J 1988, 7:949–957
10. Tanzi RE, Gusella JF, Watkins PC, Bruns GAP, St. George-Hyslop P, Van Keuren ML, Patterson D, Pajan S, Kurnit DM, Neve RL: Amyloid β-protein gene; cDNA, mRNA distributions and genetic linkage near the Alzheimer locus. Science 1987, 235:880–884
11. Goldgaber D, Lerman MJ, McBride OW, Saffiotti V, Gadjusek DC: Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 1987, 235:877–880
12. Robakis NK, Ramakrishna N, Wolfe G, Wisniewski HM: Molecular cloning and characterization of cDNA encoding the cerebral vascular and the neuritic plaque amyloid peptides. Proc Natl Acad Sci USA 1987, 84: 4190–4194
13. Van Nostrand WE, Wagner SL, Suzuki M, Choi BH, Farrow JS, Geddes JW, Cotman CW, Cunningham DD: Protease nexin-II, a potent antichymotrypsin, shows identity to amyloid β-protein precursor. Nature 1989, 341:546–549
14. Van Nostrand WE, Schmaier AH, Farrow JS, Cunningham DD: Protease Nexin-II (amyloid β-protein precursor): a platelet α-granule protein. Science 1990, 248: 745–748
15. Van Nostrand WE, Wagner SL, Shankle WR, Farrow JS, Dick M, Rozemuller JM, Kuiper MA, Wolters EC, Zimmerland J, Cotman CW, Cunningham DD: Decreased levels of soluble amyloid β-protein precursor in cerebrospinal fluid of live Alzheimer disease patients. Proc Natl Acad Sci USA 1992, 89:2551–2555
16. Sisodia SS, Koo EH, Beyreuther K, Unterbeck A, Price DL: Evidence that β-amyloid protein in Alzheimer's disease is not derived by normal processing. Science 1990, 248:492–495
17. Esch FS, Keim PS, Beattie EC, Blancher RW, Culwell AR, Oltersdorf T, McClure D, Ward PJ: Cleavage of amyloid β peptide during constitutive processing of its precursor. Science 1990, 248:1122–1124
18. Wisniewski HM, Terry RD: Reexamination of the pathogenesis of the senile plaque. Progress in Neuropathology, vol II, 2nd ed. Edited by Zimmerman HM, New York, Grune and Stratton, 1973, pp 1–26
19. Rozemuller JM, Eikelenboom P, Stam FC, Beyreuther K, Masters CL: A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J Neuropathol Exp Neurol 1989, 48:647–663
20. Wisniewski HM, Bancher C, Barcikowska M, Wen GY, Currie J: Spectrum of morphological appearance of

amyloid deposits in Alzheimer's disease. Acta Neuropathol 1989, 78:337–347
21. Braunmühl von A: Alterskrankungen des Zentralnervensystems. Senile Involution. Senile Demenz. Alzheimerkrankheit. Handbuch der spezieller pathologischen Anatomie und Histologie. Nervensystem, 2nd ed. Edited by Scholz W, Berlin, Springer, 1957, pp 337–593
22. Uyematsu S: On the pathology of senile psychosis. The differential diagnostic significance of Redlich-Fischer's miliary plaques. J Nerv Ment Dis 1923, 57:1–260
23. Braak H, Braak E, Ohm T, Bohl J: Alzheimer's disease: mismatch between amyloid plaques and neuritic plaques. Neurosci Lett 1989, 103:24–28
24. Ikeda S-I, Yanagisawa N, Allsop D, Glenner GG: Evidence of amyloid beta-protein immunoreactive early plaque lesions in Down's syndrome brains. Lab Invest 1989, 61:133–137
25. Tagliavini F, Giaccone G, Frangione B, Bugiani O: Preamyloid deposits in the cerebral cortex of patients with Alzheimer's disease and nondemented individuals. Neurosci Lett 1988, 93:191–196
26. Ikeda S-I, Yanagisawa N, Allsop D, Glenner GG: Early senile plaques in Alzheimer's disease demonstrated by histochemistry, immunocytochemistry and electron microscopy. Hum Pathol 1990, 21:1221–1226
27. Ogomori K, Kitamoto T, Tateishi J, Sato Y, Suetsugu M, Abe M: Beta-protein amyloid is widely distributed in the central nervous system of patients with Alzheimer's disease. Am J Pathol 1989, 134:234–251
28. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Ihara Y: A variety of cerebral amyloid deposits in the brains of the Alzheimer-type dementia demonstrated by beta-protein immunostaining. Acta Neuropathol 1988, 76: 541–549
29. Scholz W: Studien zur Pathologie der Hirngefässe. II. Die drusige Entartung der Hirnarterien und -capillaren. Z Neur 1938, 162:694–715
30. Divry G: De l'amyloidose vasculaire cerebrale et meningee (meningopathie amyloide) dans la demence senile. J Belg Neurol 1941, 41/42:141–158
31. Morel F, Wildi E: General and cellular pathochemistry of senile and presenile alterations of the brain. Proceedings of the First International Congress of Neuropathology, vol. II. Edited by Gozzano M. Torino, Italy, Rosenberg and Sellier, 1952, pp 347–374
32. Bahmanyar S, Higgins GA, Goldgaber D, Lewis D, Morrison JH, Wilson MC, Shankar SK, Gajdusek DC: Localization of amyloid beta protein messenger RNA in brains from patients with Alzheimer's disease. Science 1987, 237:77–80
33. Zimmermann K, Herget T, Salbaum JM, Schubert W, Hilbich C, Cramer M, Masters CL, Multhaup G, Kang J: Localization of the putative precursor of Alzheimer's disease-specific amyloid at nuclear envelopes of adult human muscle. EMBO J 1988, 7:367–372
34. Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J: Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991, 349:704–706
35. Naruse S, Igarashi S, Aoki K, Kaneko K, Iihara K, Miyatake T, Kobayashi H, Inuzuka T, Shimizu T, Kojima T, Tsuji S: Mis-sense mutation Val-Ile in exon 17 of amyloid precursor protein gene in Japanese familial Alzheimer's disease. Lancet 1991, 337:978–979
36. Yoshioka K, Miki T, Katsuya T, Ogihara T, Sakaki Y: The 717val-Ile substitution in amyloid precursor protein is associated with familial Alzheimer's disease regardless of ethnic groups. Biochem Biophys Res Commun 1991, 178:1141–1146
37. Murrell J, Farlow M, Ghetti B, Benson MD: A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science 1991, 254: 97–99
38. Luyendijk W, Bots GTAM, Vegter-van der Vlis M, Went LN, Frangione B: Hereditary cerebral hemorrhage caused by cortical amyloid angiopathy. J Neurol Sci 1988, 85:267–280
39. Wattendorff A, Bots GTAM, Went LN, Endtz LJ: Familial cerebral amyloid angiopathy presenting as recurrent cerebral haemorrhage. J Neurol Sci 1982, 55: 121–135
40. van Duinen SG, Castano EM, Prelli F, Bots GTAM, Luyendijk W, Frangione B: Hereditary cerebral hemorrhage with amyloidosis in patients of the Dutch origin is related to Alzheimer disease. Proc Natl Acad Sci USA 1987, 84:5991–5994
41. Maat-Schieman MLC, van Duinen SG, Haan J, Roos RAC: Morphology of cerebral plaque-like lesions in hereditary cerebral hemorrhage with amyloidosis (Dutch). Acta Neuropathol 1992, 84:674–679
42. Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, Bots GTAM, Luyendijk W, Frangione B: Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch-type. Science 1990, 248:1124–1126
43. van Broeckhoven C, Haan J, Bakker E, Hardy JA, Hul WV, Vegter-van der Vlis M, Roos RAC: Amyloid β-protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). Science 1990, 248:1120–1122
44. Bakker E, van Broeckhoven C, Haan J, Voorhoeve E, van Hull W, Levy E, Lieberburg I, Carman MD, van Ommen GJB, Roos RAC: DNA diagnosis for hereditary cerebral hemorrhage with amyloidosis (Dutch type). Am J Hum Genet 1991, 49:518–521
45. Tagliavini F, Ghiso J, Timmers WF, Giaccone G, Bugiani O, Frangione B: Coexistence of Alzheimer's amyloid precursor protein and amyloid protein in cerebral vessel walls. Lab Invest 1990, 62:761–766

46. Castaño EM, Ghiso J, Prelli F, Gorevic PD, Migheli A, Frangione B: *In vitro* formation of amyloid fibrils from two synthetic peptides of different lengths homologous to Alzheimer's disease β-protein. Biochem Biophys Res Commun 1986, 141:782–789
47. Gheuens J, Cras P, Perry G, Boons J, Ceuterick-de Groote C, Lübke U, Mercken M, Tabaton M, Gambetti P, Vandermeeren M, Mulvihill P, Siedlak S, Van Heuverswijn H, Martin JJ: Demonstration of a novel neurofilament associated antigen with the neurofibrillary pathology of Alzheimer and related diseases. Brain Res 1991, 558:43–52
48. Kitamoto T, Ogomori K, Tateishi J, Prusiner SB: Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. Lab Invest 1987, 57:230–236
49. Sternberger LA: Immunocytochemistry, 2nd ed. New York, John Wiley & Sons, 1981, pp 104–169
50. Puchtler H, Sweat F, Levine M: On the binding of Congo red by amyloid. J Histochem Cytochem 1962, 10:355–364
51. Shoji M, Hirai S, Yamaguchi H, Harigaya Y, Kawarabayashi T: Amyloid β-protein precursor accumulates in dystrophic neurites of senile plaques in Alzheimer-type dementia. Brain Res 1990, 512:164–168
52. Arai H, Lee VM-Y, Otvos L, Greenberg B, Lowery DE, Sharma SK, Schmidt ML, Trojanowski JQ: Defined neurofilament, tau, and β-amyloid precursor protein epitopes distinguish Alzheimer from non-Alzheimer senile plaques. Proc Natl Acad Sci USA 1990, 87: 2249–2253
53. Joachim CL, Games D, Morris J, Ward P, Frenkel D, Selkoe D: Antibodies to non-beta regions of the beta-amyloid precursor protein detect a subset of senile plaques. Am J Pathol 1991, 138:373–384
54. Ishii T, Kametani F, Haga S, Sato M: The immunohistochemical demonstration of subsequences of the precursor of the amyloid A4 protein in senile plaques in Alzheimer's disease. Neuropathol Appl Neurobiol 1989, 15:135–147
55. Cras P, Kawai M, Siedlak S, Mulvihill P, Gambetti P, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G: Neuronal and microglial involvement in β-amyloid protein deposition in Alzheimer's disease. Am J Pathol 1990, 137:241–246
56. Tate-Ostroff B, Majocha RE, Marotta CA: Identification of cellular and extracellular sites of amyloid precursor protein extracytoplasmatic domain in normal and Alzheimer disease brains. Proc Natl Acad Sci USA 1989, 86:745–749
57. Tagliavini F, Giaccone G, Verga L, Ghiso J, Frangione B, Bugiani O: Alzheimer patients: preamyloid deposits are immunoreactive with antibodies to extracellular domains of the amyloid precursor protein. Neurosci Lett 1991, 128:117–120
58. Rozemuller JM, Eikelenboom P, Kamphorst W, Stam FC: Lack of evidence for dysfunction of the blood-brain barrier in Alzheimer's disease: an immunohistochemical study. Neurobiol Aging 1988, 9:383–391
59. Cras P, Kawai M, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G: Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein. Proc Natl Acad Sci USA 1991, 88:7552–7556
60. Ko LW, Sheu KR, Blass JP: Immunohistochemical colocalization of amyloid precursor protein with cerebrovascular amyloid of Alzheimer's disease. Am J Pathol 1991, 139:523–533
61. Selkoe DJ: Molecular pathology of amyloidogenic proteins and the role of vascular amyloidosis in Alzheimer's disease. Neurobiol Aging 1989, 10:387–395
62. Kawai M, Kalaria RN, Harik SI, Perry G: The relationship of amyloid plaques to cerebral capillaries in Alzheimer's disease. Am J Pathol 1990, 137:1435–1446
63. Prelli F, Castano EM, Glenner GG, Frangione B: Differences between vascular and plaque core amyloid in Alzheimer's disease. J Neurochem 1988, 51:648–651
64. Joachim CL, Duffy LK, Morris JH, Selkoe DJ: Protein chemical and immunocytochemical studies of meningovascular β-amyloid protein in Alzheimer's disease and normal aging. Brain Res 1988, 474:100–111
65. Rozemuller JM, Abbink J, Stam FC, Hack CE, Eikelenboom P: Distribution pattern and functional state of α1-antichymotrypsin in plaques and vascular amyloid in Alzheimer's disease. Acta Neuropathol 1991, 82: 200–207
66. Shoji M, Hirai S, Harigaya Y, Kawarabayashi T, Yamaguchi H: The amyloid β-protein precursor is localized in smooth muscle cells of leptomeningeal vessels. Brain Res 1990, 530:113–116
67. Martin LJ, Sisodia SS, Koo EH, Cork LC, Dellovade TL, Weidemann A, Beyreuther K, Masters CL, Price DL: Amyloid precursor protein in aged nonhuman primates. Proc Natl Acad Sci USA 1991, 88:1461–1465
68. Cork LC, Masters CL, Beyreuther K, Price DL: Development of senile plaques. Relationships of neuronal abnormalities and amyloid deposits. Am J Pathol 1990, 137:1383–1392
69. Rozemuller JM, Stam FC, Eikelenboom P: Acute phase proteins are present in amorphous plaques in the cerebral but not in the cerebellar cortex in Alzheimer's disease. Neurosci Lett 1990, 119:75–78
70. Eikelenboom P, Rozemuller JM, Fraser H, Berkenbosch F, Kamphorst W, Stam FC: Neuroimmunologic mechanism in cerebral amyloid deposition in Alzheimer's disease. Frontiers of Alzheimer Research. Edited by Ishii T, Allsop D, Selkoe DJ, Amsterdam, Elsevier, 1991, pp 259–271
71. Kowall NW, Beal MF, Busciglio J, Duffy LK, Yankner BA: An *in vivo* model for the neurodegenerative effects of β amyloid and protection by substance P. Proc Natl Acad Sci USA 1991, 88:7247–7251