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β-Amyloid Protein of Alzheimer's Disease Is Found in Cerebral and Spinal Cord Vascular Malformations

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Congo/Red deposition with birefringence to polarized light was demonstrated focally in cerebrovascular malformations removed surgically from 4 older patients (ages 85, 74, 74, and 63), and in a spinal cord vascular malformation in a 76-year-old patient. Lesser degrees of Congophilic change were observed in cerebrovascular malformations screened from 4 of 10 patients between the ages of 30 and 59. No Congophilic change was seen in 10 cerebrovascular malformations removed from patients under 30 years of age. Congophilic areas in all cases decorated with W-2 and 85/45 polyclonal antibodies raised to peptide sequences of cerebrovascular β-amyloid and β-amyloid of senile plaques from patients with Alzheimer's disease. Thus, the amyloid in these vascular malformations is immunologically related to β-amyloid protein. This finding provides another indication that vascular β-amyloid deposition is not specific for Alzheimer's disease and suggests that an existing abnormality of vessels may be a predisposing factor. (Am J Pathol 1988, 132:167–172)

CEREBROVASCULAR AMYLOID is commonly found in association with senile dementia of the Alzheimer type (SDAT) and also in elderly persons without dementia.[1,2] Both groups of patients are at risk for cerebral hemorrhage.[1] Cerebrovascular amyloid is referred to as β-amyloid protein and it is identical or nearly identical to the amyloid found in the cores of the senile plaques in SDAT.[3] Dutch families have been reported who have hereditary cerebrovascular amyloidosis associated with cerebral hemorrhage. In these patients the amyloid has been identified as β-amyloid protein.[4] Icelandic families have also been reported to have hereditary cerebral hemorrhage with amyloidosis but in these cases the amyloid is composed of a variant of cystatin C.[5] Recently the DNA encoding β-amyloid protein has been isolated, cloned, and localized to chromosome 21, in the same region where the gene for familial Alzheimer's disease maps.[6,7] This linkage could indicate that β-amyloid formation is a critical factor in the expression of SDAT. In addition to the expression of a gene that codes for the β-amyloid protein, however, other factors are obviously necessary for the expression of SDAT. Some of these factors may have to do with vascular conditions that allow for the deposition of β-amyloid protein in the brain, or β-amyloid formation in SDAT could simply be an epi-phenomenon.

It is the purpose of this report to show that vascular malformations of the central nervous system can contain amyloid and that this amyloid is immunologically similar to the β-amyloid protein found in the blood vessels and senile plaques of persons with SDAT. Further, the amount of the deposited amyloid in vascular malformations appears to be directly proportional to the age of the patient. One possible interpretation of these observations is that circulating β-amyloid proteins require preexisting abnormal vessels as a condition for deposition and that their deposition is slowly cumulative over long periods of time.

Materials and Methods

Materials

Eighty-five surgically removed cerebrovascular malformations were collected from the files of the

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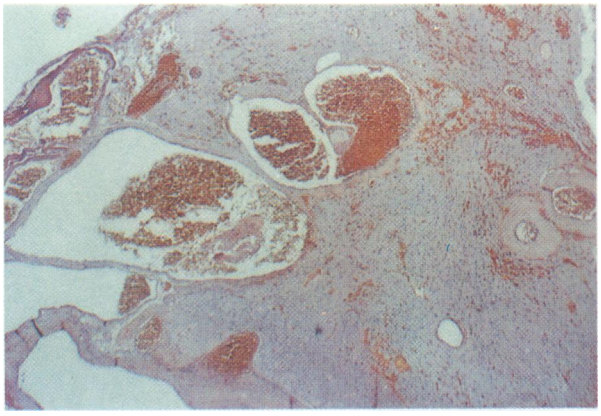
University of Iowa. The mean age of the patients at the time of surgery was 36 and the median age was 37 with a range of 2 months to 85 years. Fifty-eight percent were men and 42% women. Ten of the malformations removed from 36 patients in the 2 months to 29 years age range were screened for amyloid deposition and 10 of 40 malformations from the 30–59-year-old group were also screened. All 9 cases in the 60–85-year-old group were screened. None of these patients were demented at the time of surgery. Initial screening was performed with Lillie and Fullmer's modification[8] of the Bennhold Congo red stain[9] on 10-μ thick sections. Additional histochemistry, electron microscopy, and immunohistochemistry were performed on the lesions of 4 patients from the older age group showing the most amyloid (ages 85 [patient A], 74 [patient B], 74 [patient C] and 63 [patient D]). A diagnosis of cerebrovascular malformation was made by angiogram and confirmed by tissue examination in 3 of the cases and by microscopic examination of the surgical specimen alone in a fourth. All 4 of these cases involved meninges and gray matter. One extended through the white matter to the ventricle and another probably extended partly into the white matter. In addition, a fifth case is included in this study was an intradural, extra parenchymal spinal cord malformation at T-11 to T-12 in a 76-year-old patient (patient E), which was diagnosed by angiogram and histologically. The Highman alkaline Congo red stain[10] with and without potassium permanganate oxidation was performed,[11] followed by visualization under standard polarizing filters with a Leitz microscope.

In 1 of the 5 cases a Congophilic region of the vessel was examined by electron microscopy following deparaffinization and reembedding in epoxy resin. Sections of the malformed vessels from all 5 of the older patients (A, B, C, D, and E) were stained by the avidin–biotin immunoperoxidase method[12] for prealbuminin, fibrinogen, IgA, IgD, IgG, IgM, kappa, and lambda to determine whether there was nonspecific trapping of serum proteins in the abnormal vessels and to enable us to compare our findings with those of other studies of cerebrovascular amyloid.[13,14] Rabbit anti-prealbumin (Behring Diagnostics, LaJolla, CA), fibrinogen, IgA, IgD, IgG, IgM, kappa, and lambda (Dako, Santa Barbara, CA) were employed as the primary antibodies. Briefly, the tissue sections were deparraffinized and the endogenous peroxidase activity blocked by incubation in 0.3% H_2O_2 in methanol for 30 minutes. To minimize nonspecific background staining the sections were then incubated with 4% normal goat serum diluted in Tris buffer (pH, 7.6). Sections were then incubated with the primary antisera (dilutions of 1:40, 1:80, 1:160, and 1:320 in 1% normal goat serum were employed for each antibody) for 60 minutes at room temperature. After rinsing, sections were incubated with diluted biotinylated goat anti-rabbit antibody for 30 minutes at room temperature. Following rinsing, sections were incubated with the avidin-biotinylated horseradish peroxidase complex reagent (Vector Labs, Burlingame, CA), rinsed again in buffer, and finally incubated with amino ethyl-carbazole (AEC) substrate to achieve color reaction.

Sections of malformations were also stained with W-2 and 85/45 antisera using an indirect peroxidase method. The W-2 antisera was raised in rabbits to the 1-24 amino acid synthetic peptide of Glenner's sequence for a peptide isolated from human cerebrovascular amyloid. It has been shown to react with Congophilic vessels and neuritic plaques from SDAT patients but does not react with neurofibrillary tangles or scrapie amyloid.[15,16] The 85/45 antisera was a gift from Colin Masters of Perth, Australia, and was raised in rabbits to a synthetic peptide of amino acids 11-23 of his sequence to the amyloid in neuritic plaque cores of SDAT.[3,17] The sequence is the same as the one derived from Congophilic cerebrovascular vessels by Glenner resulting in the W-2 antisera.[15,16] The 85/45 antisera has been shown to react with SDAT Congophilic vessels and the amyloid component of senile neuritic plaque cores. Both W-2 and 85/45 were used in 1:125, 1:250, and 1:500 dilutions. The staining protocol was the same as that listed in the above paragraph with the exception that avidin-labeled peroxidase was used instead of the ABC complex and the slides were developed in diaminobenzidine. Autopsy sections of brain from cases of SDAT with Congophilic angiopathy (positive control) and renal glomerular amyloid deposition associated with myeloma (secondary amyloid; negative control) were used as controls for both W-2 and 85/45.

Results

None of the 10 cerebrovascular malformations from the younger patients showed any Congophilia. Four of the 10 malformations (Figure 1) screened from the 30–59-year-old age group showed focal areas of Congophilic change which were also birefringent under polarized light. All 9 of the lesions from patients above 60 years of age showed Congophilic changes somewhere in the malformed vessels. The most significant changes in terms of area involved and intensity of Congo red staining were seen in 4 of the 5 older patients, with the greatest amount of amyloid clearly seen in the oldest (patient A, 85 years). The Congophilic changes were found in hyalinized areas of both media and adventitia of the abnormal vessels and tended to be focal. Numerous other areas of hyalinization



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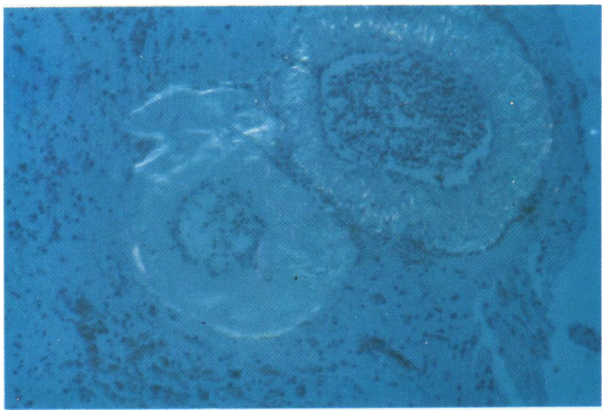
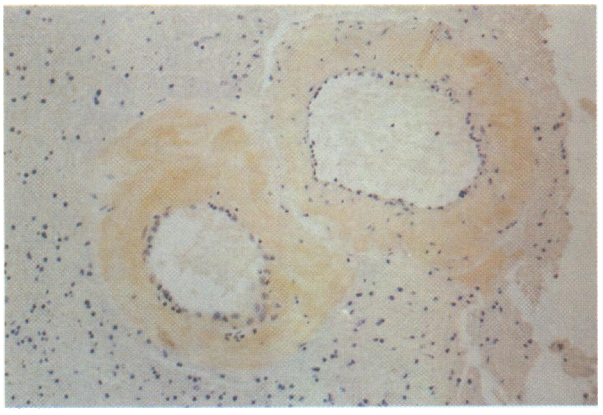


2A



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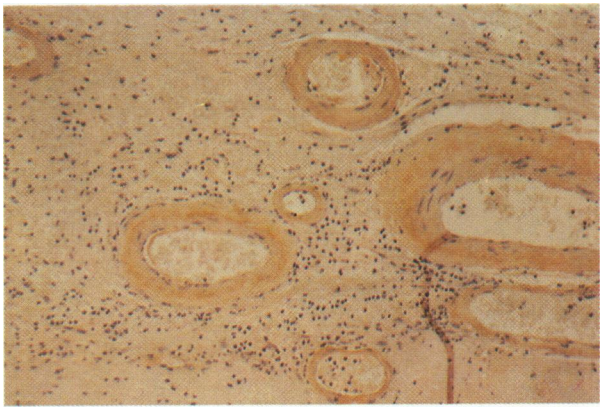

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Figure 1—Cerebral arteriovenous malformation from the 63-year-old patient, typical of the type seen in all 4 older patients. (H & E, × 16) **Figure 2A**—Abnormal vessels from the malformation in the 76-year-old patient under polarized light exhibiting green birefringence. These are the same vessels shown on the far right side of Figure 1. (Congo red stain, × 100) (Editor's note: due to variations in color separation, the light areas in Figure 2A do not show the apple-green birefringence of the original photomicrograph.) **Figure 2B**—Section adjacent to 2A showing staining with the 85/45 antibody in the same areas polarized. (1:250 dilution of primary antibody; × 100) **Figure 3**—A cluster of abnormal vessels from patient B showing strong staining with the W-2 antibody. (1:250 dilution of primary antibody; × 100)

both within and extraneous to vessel walls, characteristic of cerebrovascular malformations, were negative for the Congo red stain. Most of the vessels displaying Congophilic change did not have elastica. Typically, small, satellite vessels adjacent to the main mass of tangled vessels were also Congophilic. Examination of material from the 1 malformation that extended to the ventricle showed extensive Congophilic change but it could not be determined which part of the malformation was cortical and which was white matter because the malformed vessels were surrounded only by reactive, gliotic tissue. In all cases the areas that were Congophilic also displayed "apple-green" birefringence by polarized light that was often contrasted with adjacent orange dichroism (Figure 2A). Polarization of a different tinctorial quality emanated from collagen in the abnormal vessel walls. Congophilia was retained after potassium permanganate treatment in sections of lesions from all 5 patient's lesions.

All 5 patient's lesions stained with the W-2 antisera and 3 of the 5 stained with the 85 45 antisera in all 3 dilutions. Most of the staining coincided precisely with Congophilic changes (Figures 2A, 2B). The staining was not intense in most areas but overall was of the same intensity as the control slides of Congophilic vessels from the SDAT patient (not shown). There was occasional staining of either W-2 or 85 45 in areas not demonstrably Congophilic and some Congophilic areas did not decorate with the antisera. The W-2 antibody generally stained more heavily than the 85/45 (Figure 3). Because of the tortuous condition of these malformed vessels, it was difficult to obtain serial sections of a quality suitable for application of all the stains and immunohistochemical procedures to precisely the same areas. Nonetheless, in the lesion sites where this was accomplished, there was excellent geographic correlation of the findings, particularly between Congophilia and W-2 and 85/45 reactivity. Immunohistochemical stains for pre-albumin, fibrinogen, kappa and lambda chains, IgM, IgA, IgD, and IgG were negative at all dilutions. Neither W-2 nor 85/45 stained the renal glomerular amyloid. Electron micrographs from a Congophilic area of patient D's cerebral vessels showed 9–12 nm nonbranching, non-

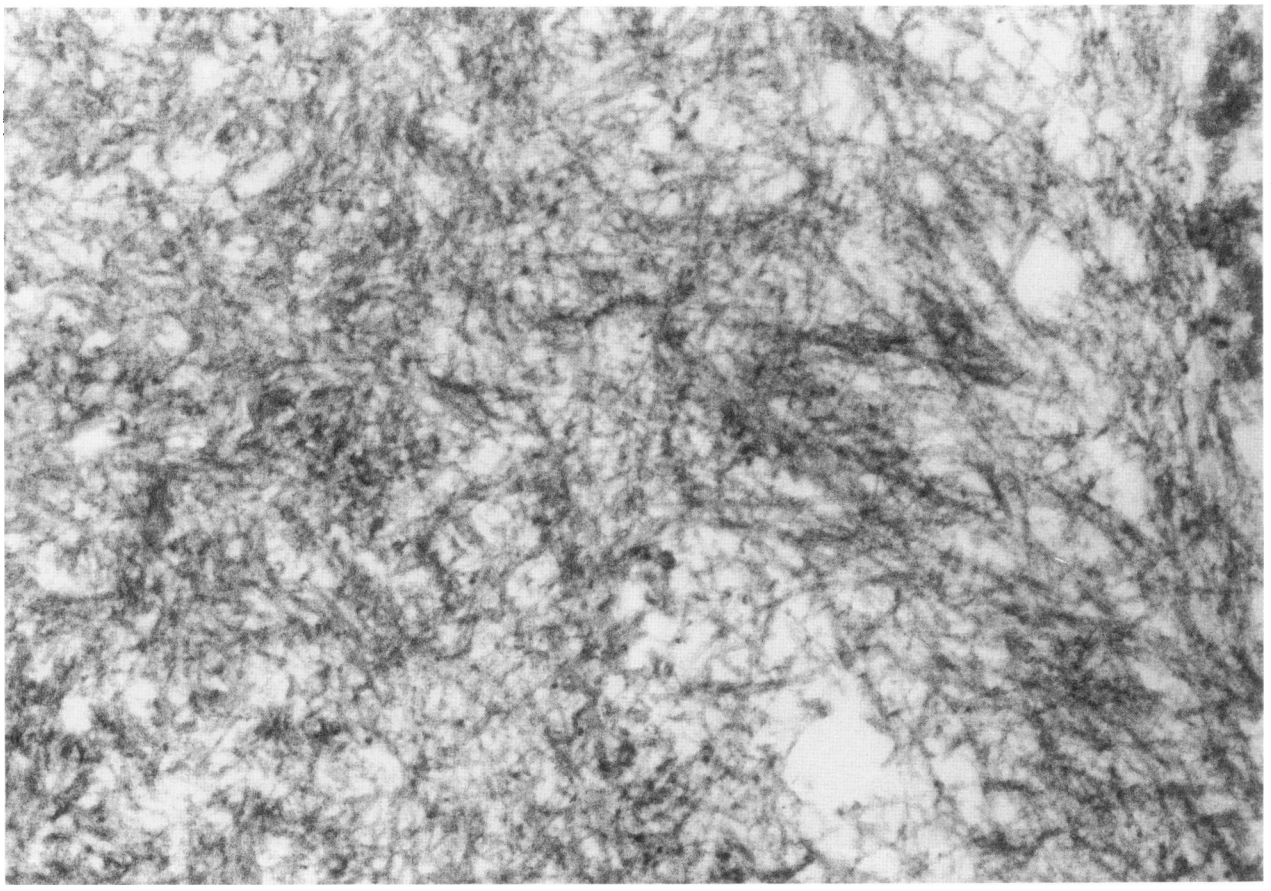

Figure 4—Electron micrograph of typical amyloid fibrils from the wall of an abnormal vessel in the 85-year-old patient. (×56,000)

parallel filaments interdigitating in an arrangement characteristic of amyloid and replacing the smooth muscle of the vessel wall (Figure 4).

In 2 of the specimens (from patients B and D), small pieces of adjacent, normal appearing cerebral cortex were included. No abnormal or Congophilic vessels were seen in these and there were neither neuritic plaques nor neurofibrillary tangles by modified Bielschowsky silver stains. Patient B (76-years-old) was followed for 3.5 years after surgery and had a spastic gait but no evidence of dementia. Patient C was followed for 5 years after surgery and had no decrease in mental function. Patient E was normal prior to surgery but was left akinetic and mute following surgery. She has shown significant improvement and at a clinic visit 2-years after surgery was noted to speak fluently with normal language and showed no evidence of compromised intellectual function. Patient D has been lost to follow-up. Patient A died within 6 months of his surgical procedure but no autopsy was performed.

Discussion

Cerebrovascular malformations are unusual in elderly persons. In the present study an age-related incidence of Congophilic change was observed in 13 of 29 vascular malformations screened for amyloid. All 9 patients over the age of 60 years with cerebrovascular malformations demonstrated Congophilia in the malformed vessels. Among these 9 patient's lesions, the 5 that appeared to have the greatest amount of amyloid compared with the others were selected for immunohistochemical studies. None of these 5 lesions stained for normal serum proteins or pre-albumin in contrast to observations reported for vessels and senile plaques in SDAT.[14,18] However, some investigators think that the presence of serum proteins in association with cerebrovascular amyloid is the result of nonspecific vascular leakage.[18-20] Thus, we do not think that our failure to stain for proteins in these vascular malformations is of any significance. All lesions remained Congophilic after treatment of the histologic sections with potassium permanganate, indicating that the amyloid was not of the secondary type.[11] Most significantly Congophilic areas in all of the lesions also stained with either 1 or both of 2 antisera raised against synthetic peptide sequences derived from cerebrovascular and neurofibrillary core amyloid from humans with SDAT disease.[3,15,16] This indicates that the amyloid present in elderly persons' cerebrovascu-

lar malformations is similar or identical to the β-amyloid protein found in the brains of patients with SDAT.

To our knowledge only 1 other case of amyloid deposition in a cerebrovascular malformation has been reported and that was in a 73-year-old man.[21] Thus, the suggestion is offered that the amyloid protein may accumulate for very long periods of time before it reaches sufficient concentration for detection. This suggestion is corroborated by the findings that amyloid deposition in senile plaques and vessels of humans with and without frank SDAT is roughly proportional to age.[1,22-24] The same appears to be true of animals.[23]

Of prime interest is the fact that none of the 5 older patients selected had dementia at the time of surgery, and in 2 of the excised lesions adjacent normal cortical tissue revealed no evidence of either Congophilic vessels or neuritic plaques. Two to 5-year follow-up in 3 of the 5 patients revealed no obvious dementia or decrease in cognitive functions and strong statements to that fact were written in their charts following clinic visits, although formal testing was not performed. Thus, these 3 patients did not have frank SDAT although it is still possible that SDAT was present in sub-clinical form.

If none of these patients had β-amyloid deposition in normal vessels then it could be argued that perhaps vessels need to be altered in some fashion to invite β-amyloid deposition. This is conjecture, but Mandybur and Gore's report[26] of amyloid in cerebral vessels following radiation therapy also invites such speculation. Similarly, amyloid deposition in vessels has been reported in association with granulomatous arteritis[27,28] as well as with cerebral vasculitis associated with rheumatoid arthritis,[29] all in older persons. However, whether it was β-amyloid deposited in these cases was not determined. Vinters[1] reports a 30% overlap between Congophilic amyloid angiopathy and clinical hypertension but there is no evidence to suggest any cause and effect relationship.

Deposition of amyloid in the walls of both large and small abnormal vessels in our cases and particularly in the extramedullary region of the spinal cord suggests that perhaps the abnormal β-amyloid or it's precursor arrives at these vessels via the systemic circulation. This suggestion is consistent with the findings of Tanzi et al,[17] who demonstrated significant quantities of mRNA for β-amyloid protein in all tissues examined. The findings are not as consistent with the hypotheses offered by Goldgaber et al,[6] who said that vascular amyloid deposits result from amyloid production by microglial cells or brain macrophages; or with the findings of Bahmanyar et al that messenger RNA for β-amyloid protein is present in neurons.[30] On the other hand, because true macrophages found in the brain arrive from the systemic circulation,[31] they might be the vehicle for amyloid delivery to brain vessels. It is also possible that other necessary factors emanating from intrinsic brain cellular constituents process the β-amyloid protein to enhance its deposition. Thus, the problem of the origin of cerebrovascular β-amyloid is not settled. Also not settled is the issue of whether the amyloid found in neurofibrillary tangles is of the β-protein type.[32]

The demonstration that the gene for β-amyloid is located on chromosome 21 in proximity to the autosomal dominant gene defect associated with familial Alzheimer's disease conveys the suggestion that the production of β-amyloid protein may be an important aspect of the pathogenesis of Alzheimer's disease.[6,7] More recent findings indicate, however, that there is no close linkage between the genetic defect in familial Alzheimer's disease and the β-amyloid gene.[33,34] The present findings also suggest dissociation between an Alzheimers gene and a β-amyloid gene because they show that β-amyloid proteins can be deposited in abnormal vessels in the brains or spinal cords of otherwise normal persons without the usual hallmarks of Alzheimer's disease. This is consistent with the recent discovery that messenger RNA encoding β-amyloid protein is found in various cortical neurons in brains from normal humans, patients with SDAT, and monkeys[30] and also is consistent with our hypothesis that whatever the source of the β-amyloid, it may require a substrate such as an altered blood vessel for deposition. It may also be important that β-amyloid was found in a spinal cord malformation. This suggests that there is no intrinsic quality of cerebral tissue that invited β-amyloid deposition.

That the β-amyloid protein was found in greatest abundance in the malformations of our oldest patients suggests that the β-amyloid protein gene is either "turned-on" in later life or that it takes a long time for amyloid to accumulate. Either way, the implication is that neither gene expression of β-amyloid protein nor its deposition in abnormal vessels explains the pathogenesis of Alzheimer's disease.

References

1. Vinters AV: Cerebral amyloid angiopathy: A critical review. Stroke 1987, 18:311-324
2. Glenner GG: Alzheimer's disease: The commonest form of amyloidosis. Arch Pathol Lab Med 1987, 107: 281-286
3. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K: Amyloid plaque core protein in Alzheimer disease and Down's Syndrome. Proc Natl Acad Sci 1985, 82:4245-4249
4. VanDuinen SG, Castano EM, Prelli F, Bots GTAB, Luyendijk W, Frangione B. Hereditary cerebral hemor-

rhage with amyloidosis in patients of Dutch origins is related to Alzheimer disease. Proc Natl Acad Sci 1987, 84:5991–5994
5. Lofberg H, Grubb AO, Nilsson EK, Jensson O, Gudmundsson G, Blondal H, Arnason A, Thorsteinsson L. Immunohistochemical characterization of the amyloid deposits and quantitation of pertinent cerebrospinal fluid proteins in hereditary cerebral hemorrhage with amyloidosis. Stroke 1987, 18:431–440
6. Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC: Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 1987, 235:877–880
7. Tanzi RE, Gusella JF, Watkins PC, Bruns GAP, St. George-Hyslop P, Van Keuren ML, Patterson D, Pagan S, Kurnit DM, Neve RL: Amyloid β protein gene: cDNA, mRNA distribution and genetic linkage near the Alzheimer locus. Science 1987, 235:880–884
8. Lillie RD, Fullmer HM: Histopathologic technique and practical histochemistry. New York, McGraw-Hill, 1976 pp 663–666
9. Bennhold H: Eine spesifische amyloid fäbung mit kongarot. Munche Med Wchnschr 1922, 69:1537–1538
10. Highman B: Improved methods for demonstrating amyloid in paraffin sections. Arch Pathol 1946, 41:559–562
11. Van Rijswijk MH, Van Heusden CWG: The potassium permanganate method: A reliable method for differentiating amyloid AA from other forms of amyloid in routine laboratory practice. Amer J Pathol 1979, 97:43–54
12. Hsu SM, Paine L, Fanger H: Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem 1981, 29:577–580
13. Kitamoto T, Tashima MD, Tateishi J: Novel histochemical approaches to the pre-albumin-related senile and familial forms of systemic amyloidosis. Am J Pathol 1986, 123:407–412
14. Shirahama T, Skinner M, Westermark P, Rubinow A, Cohen AS, Brun A, Kemper TL: Senile cerebral amyloid: Prealbumin as a common consistuent in the neuretic plaque, in the neurofibrillary tangle and in the microangiopathic lesion. Am J Pathol 1982, 107:41–50
15. Wong CW, Quaranta V, Glenner GG: Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related. Proc Natl Acad Sci 1985, 82: 8729–8732
16. 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
17. 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
18. Torack RM, Lynch RG: Cytochemistry of brain amyloid in adult dementia. Acta Neuropathol (Berl) 1981, 53:189–196
19. Goust JM, Mangum M, Powers JM: An immunologic assessment of brain associated IgG in senile cerebral amyloidosis. J Neuropathol Exp Neurol 1984, 43:481–488
20. Eikelenboom P, Stam FC: An immunohistochemical study on cerebral vascular and senile plaque amyloid in Alzheimer's dementia. Virchows Arch (Cell Pathol) 1984, 47:17–25
21. Peterson EW, Schulz DM: Amyloid in vessels of a vascular malformation in brain. Arch Pathol 1961, 72: 118–121
22. Wright JR, Calkins E, Breen WJ, Stolte G, Schultz RT: Relationship of amyloid to aging. Medicine 1969, 48: 39–60
23. Morris JH, Joachim CL, Selkoe DJ. A case of Alzheimer-like amyloid deposits without Alzheimer's disease (Abstr). J Neuropathol Exp Neurol 1987, 46:338
24. Nadeau SE, Bebin J, Smith E. Nonspecific dementia, cortical blindness, and congophilic angiopathy. J Neurol 1987, 234:14–18
25. Selkoe DJ, Bell DS, Podlisny MB, Price DL, Cork LC: Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. Science 1987, 235:873–877
26. Mandybur TI, Gore I: Amyloid in late postirradiation necrosis of brain. Neurology 1969, 19:983–992
27. Murphy MN, Sima AF: Cerebral amyloid angiopathy associated with giant cell arteritis: A case report. Stroke 1985, 16:514–517
28. Probst A, Ulrich J: Amyloid angiopathy combined with granulomatous angiitis of the central nervous system: Report on two patients. Clinical Neuropath 1985, 4: 250–259
29. Mandybur TI. Cerebral amyloid angiopathy: Possible relationship to rheumatoid vasculitis. Neurol 1979, 29: 1336–1340
30. Bahmanyar S, Higgins GA, Goldgaber D, Lewis D, Morrison JH, Wilson MC, Shankar SK, Gajdusek DC: Localization of amyloid β protein messenger RNA in brains from patients with Alzheimer's disease. Science 1987, 237:77–80
31. Schelper RL, Adrian EK: Monocytes become macrophages; they do not become microglia: A light and electron microscopic autoradiographic study using 125-Iododeoxyuridine. J Neuropathol Exp Neurol 1986, 45: 1–19
32. Selkoe D, Abraham CR, Podlisny MB, Duffy LK: Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. J Neurochem 1986, 46:1820–1834
33. Broeckhoven CV, Genthe AM, Vandenberghe A, Horsthemke B, Backhovens H, Raeymaekers P, Van Hul W, Wehnert A, Gheuens J, Cras P, Bruyland M, Martin JJ, Salbaum M, Multhaup G, Masters CL, Beyreuther K, Gurling HMD, Mullan MJ, Holland A, Barton A, Irving N, Williamson R, Richards SJ, Hardy JA: Failure of familial Alzheimer's disease to segregate with the A4-amyloid gene in several European families. Nature 1987, 329:153–155
34. Tanzi RE, St George-Hyslop PH, Haines JL, Polinsky RJ, Nee L, Foncin JF, Neve RL, McClatchey AI, Conneally PM, Gusella JF. The genetic defect in familial Alzheimer's disease is not tightly linked to the amyloid B-protein gene. Nature 1987, 329:156–157