APOE polymorphism and clinical duration determine regional neuropathology in Swedish APP_{670,671} mutation carriers: implications for late-onset Alzheimer's disease

N. Bogdanovic^{a*}, Elizabeth Corder^b, L. Lannfelt^a, B. Winblad^a

^a *NEUROTEC, Section of Geriatric Medicine, Karolinska Institute, Stockholm, Sweden*

^b *Center for Demographic Studies, Duke University, Durham, North Carolina, USA*

Received: May 30, 2002; Accepted: June 15, 2002

Abstract

Neurofibrillary changes throughout the brain were investigated for three relatives who carried the Swedish APP_{670,671} mutation which causes overproduction of A β 40 and A β 42. They differed in terms of APOE genotype, age at the onset of dementia, and disease duration (P1: ϵ 2/3, age 57, 11 years; P2: ϵ 2/3, age 61, 5 years; P3: ϵ 4/4, age 44, 12 years). For each subject, paraffin-embedded sections from diverse anatomically and cytoarchitectonically well-preserved regions were stained using the modified Bielschowsky method. Neurofibrillary tangles (NFT) and neuritic plaques (NP) were counted, and the area occupied by plaque estimated (%NP). In addition, sections from the medial frontal gyrus were stained with monoclonal antibodies to APOE. The regional patterns of neurofibrillary changes were consistent with those for late-onset AD. Longer disease duration was associated with further accumulations in earlier-affected areas, with superficial cortical layers consistently containing higher %NP than deep layers. APOE ϵ 4/4 was associated with deeper limbic and frontal NFT, with an excess of NP (especially in the outer parietal cortex) which stained heavily for APOE - as well as with very early onset. APP_{670,671} mutation carriers demonstrate regional brain neurofibrillary changes characteristic of late-onset Alzheimer's disease with evidence for more A β deposition for ϵ 4/4 than ϵ 2/3. This raises the possibility that early Braak Stage I-II lesions might also follow this pattern of promotion.

Keywords: Alzheimer • Swedish mutation • APOE • early-onset • quantification

Introduction

Detailed comparisons of Alzheimer's disease (AD) pathology in rare dominant mutation carriers (usually affected in mid-life) and in common late-

onset AD have not been made. Three relatives here called P1, P2, P3 who carried the Swedish APP_{670,671} mutation (which causes overproduction/deposition of A β 40 and A β 42) [1-5] were investigated using a structured anatomical approach to quantifying regional brain neurofibrillary changes to facilitate such comparisons and demonstrate the approach.

* Correspondence to: Nenad BOGDANOVIC, MD, PhD
NEUROTEC, Geriatric Section, KI, NOVUM, KFC, level 4
S-14186, Stockholm, Sweden.
Tel: +46-8-585 83888, Fax: +46-8-585 83880
E-mail: brainbank@kfc.hs.sll.se

Similar patterns would etiologically link the disorders, directly involving amyloid overproduction and deposition in late-onset AD.

The trait has been traced through eight generations for a single Swedish family. For 26 documented subjects, age at onset varied from 44 to 61 years (mean, 53 years) and duration from the clinical onset of dementia until death ranged from 3 to 13 years (mean, 8.5 years) — similar to carriers of APP₇₁₇ and PS2 mutations. Presenting symptoms are deficits in memory and abstract reasoning, although four subjects including P3 presented with psychiatric symptoms (depression, paranoid misconceptions, aggressiveness) [6, 7].

Previous autopsy series in general samples demonstrate a sequenced spreading of neurofibrillary pathology over decades, from early adulthood onset in the transentorhinal cortex, across the hippocampus to the cortex, symmetrically within both hemispheres — with isocortical changes appearing first in the temporoparietal cortex [8-11]. Whether further accumulation in already-affected regions continues in the clinical stages of AD is not known. Contrasting P1 with P2, having 11 and 5 years expression of dementia respectively, may offer a glimpse of accumulation in the clinical stages of AD assuming that both disorders follow the same patterns of neurodegeneration. These subjects were brothers, had extensive gene sharing, carrying the low-risk-for-AD ϵ 2/3 APOE genotype, and had similar ages at onset (57 and 61 years).

Distant cousin P3 (onset at age 44, 12 years duration) carried the highest-risk-for-AD APOE ϵ 4/4 genotype also allowing investigation of altered regional spread of neurofibrillary changes

by comparison with P1. This possibility is suggested by reports indicating that the ϵ 4 isoform binds A β more rapidly *in vitro* than ϵ 3 [12,13] and speeds formation of amyloid fibrils when mixed with soluble A β [14-16]. APP₇₁₇ [17] and PS2 [18-19] mutation carriers and Down's syndrome patients [20,21] show altered APP processing and earlier clinical onset for ϵ 4, not found for PS1 [22,23]. Clearly APOE ϵ 4 gene dose shifts late-onset AD risk to younger ages [24].

Isoform-specific differences in AD progression may be minor [25, 26] despite greater cortical amyloid load for a given duration of symptoms when ϵ 4 is present [27-31]. The subset of APP_{670,671} carriers presenting with psychiatric symptoms, including P3 carrying ϵ 4/4, intriguingly hint that ϵ 4 might alter the pattern of AD pathogenesis as well as increase AD risk [32, 33].

A structured anatomical approach and APOE immunostaining were employed to compare regional brain pathology for APP_{670, 671} carriers P1, P2, and P3. The goals were to investigate the effects of dementia duration and APOE polymorphism on neurofibrillary changes and to compare these subjects with previous reports concerning late-onset AD cases.

Material and methods

Study subjects

Information on P1, P2 and P3 is summarized in Table 1. The diagnosis of definite AD was established independently by two neuropathologists according to CERAD clinical and neuropathological criteria [34-36]. Clinical onset was estimated from each patient's complete medical record supplemented with

Table 1. Information on P1, P2 and P3 subjects.

Patients	Sex	Presenting disturbances	Onset age (years)	Age at death (years)	Disease duration (years)	APOE genotype
P1	M	memory	57	68	11	ϵ 2/3
P2	M	memory	61	66	5	ϵ 2/3
P3	M	paranoia memory	44	56	12	ϵ 4/4

Table 2. Brain regions.

Area	Level
Gyrus frontalis medialis	genu corporis callosi
Gyri orbitofrontalis	genu corporis callosi
Gyrus prepolaris	top of the lobe, 1 cm from medial line
Gyrus pre-/postcentralis	presentation of the hand
Gyrus supramarginalis	dorsal end of sulcus temp sup
Occipital cortex I&II	2 cm rostral from occipital pole
Gyrus cinguli	1.5 cm posterior from gen.corp.call.
Gyri Temp superior/medius	corpus mamillare
Enorhinal cortex	corpus mamillare
Amygdala	commissura anterior
Periaqueductal gray	nucleus oculomotorius
Hippocampus anterior	corpus mamillare
Hippocampus posterior	corpus geniculatum laterale
Cortex cerebelli	right (lobulus simplex)
Nucleus basalis Meynert	commissura anterior (post. edge)
Basal ganglia	commissura anterior (post. edge)

information from relatives. The subjects died from bronchopneumonia and/or cardiovascular insufficiency.

Fixation, dissection and staining

Brain tissue was processed within 24 hours of death using the strict protocols for fixation, dissection, histochemistry and quantitation used for all Huddinge Brain Bank samples (internal protocol). Tissue was fixed in buffered 4% formaldehyde for 4-6 weeks, paraffinized and sectioned (8µm thick). The Bielschowsky staining method [37] was used to allow quantitation of neurofibrillary pathology.

Shrinkage

An important preliminary part of the project was estimating tissue shrinkage due to fixation, paraffinization, and staining. There was 5% expansion after two weeks of fixation; shrinkage to prefixation volume at 4-6 weeks (irrespective of brain size and region); and continued shrinkage thereafter. Given the lack of shrinkage at 4 to 6 weeks, when paraffinization was begun, we did not estimate a shrinkage factor (SF) for fixation. SF for paraffinization and staining were estimated as follows for 15 tissue blocks (5 from each

subject): A point-grid blade with regularly spaced intervals was placed on top of the block surface to be sectioned and stained, i.e. after fixation and, again, after paraffinization and staining. The number of grid-points encompassed by the surface was counted. The SF for paraffinization and after staining were 23% and 11%, respectively. Thus, all measurements were corrected for 11% shrinkage.

Quantification

AD pathology was quantified using the Olympus Video Stereological Analysis System (BICO, Copenhagen). The system involves a SONY color CCD video camera mounted on a Zeiss Axioskop microscope. A Sony camera adapter CMA-D2 communicated images to the color monitor of an IBM PC (Pentium). GRID v2.0 software (Interactivision ApS, Silkeborg) was used to generate counting frames of specified dimension (for superimposition on the screen image), and to control the x and y movement of the microscopic stage by two Multicontrol 2000 programable stepping motors.

The number of NFT, NP and DP per square millimeter was counted within superficial layers 1-3 and deep layers 4-6 for 11 cortical areas and for 12

subcortical structures (Table 2). A particular region was first outlined on the screen at low magnification (x4) using the cursor. Then a 100x oil-immersion objective with a numerical aperture of 1.40 was moved into place and the counting frame superimposed. Fields with preserved cytoarchitectonic characteristics and no tangentially cut structures suggesting penetrating arteries from the cortical surface were selected. An estimate of the numerical density of NFT, NP and DP within a section area was achieved with approx. 30 observations [38].

The areas of the counting frames were dimensioned so that 3-4 NFT, and 1-2 NP, were often sampled. Systematic random counting frame samples were obtained by stepping, using the meander path function of GRID. NFT and NP were counted when they were within the frame or crossed exactly two free but constantly-defined edges of the frame. Three replicate measurements were made. For the cortex, fields in the top, middle and bottom of the gyrus were sampled.

Plaque load (%NP) was estimated as the ratio between the number of positive grid points (hits) encountering plaque formations to the total number of points in the area. Hence two-dimensional NP profiles were used to approximate plaque area occupancy. Diffuse plaques (DP) are reported only in the regions where neurofibrillary changes could not be discerned. Note: In the sense of stereological theory and two-dimensional quantification the terms NP-profiles and NFT-profiles are more correct. For simplicity the profiles are referred to as NFT or NP counts and %NP load.

APOE genotyping and immunostaining

DNA was extracted from peripheral white blood cells using standard methods. APOE genotype was determined using a micro-sequencing method on microtiter plates (AffiGen APOE™, Sangtec Medical, Bromma, Sweden) [39].

Eight µm thick sections from the medial frontal gyrus (layers 1-6) of each subject were taken for indirect immunostaining with mouse APOE monoclonal antibodies (MAb) (Boehringer Mannheim, Germany) according to Blennow [40]. Briefly, APOE antibodies (0.5 µg/ml) were applied to the sections, incubated at 40C overnight, treated with biotinylated horse anti-mouse antibody (3 µg/ml, Vector Laboratories Burlingame, UK) for 30 minutes, followed by incubation in ABC-Elite HRP (Vector Laboratories) for 1 hour and visualised by use of a DAB-nickel chloride enhancing procedure. The MAb had equal affinity for each common APOE isoform. For control staining the primary antibody was omitted.

Statistical methods

The number of NFT and NP, and the %NP, were compared for superficial *versus* deep cortical layers separately for each subject. The effects of duration (P2 - P1) and APOE polymorphism (P3 - P1) were also investigated. Comparisons employed Wilcoxon's signed rank and Kruskal-Wallis tests, implemented using SAS procedures FREQ and NPAR1WAY (SAS Institute). The sample size was 66 for comparisons involving the cortex: 11 regions with 6 independent comparisons among the replicate measurements.

Results

Overview

For each subject, NFT were abundant in the hippocampus (Fig. 1 a) (green-gray), and tended to be more common in the superficial layers 1-3 (red) compared to the deep cortical layers (red + horizontal bars). NP and %NP were abundant in the superficial cortex, less common in the deeper layers and least common in the hippocampus (Fig. 1 b-c). The means for a number of areas are shown: hippocampus — CA1-CA4 areas, dentatus and subiculum; cortex — superior temporal, entorhinal, postcentral, supramarginal, prefrontal, medial, orbitofrontal, precentral, anterior cingulate, primary and secondary visual areas.

AD duration and APOE genotype

Despite the overall similarity for the three subjects, shorter duration of disease was associated with less accumulation of AD pathology. Specifically, P2 had statistically significantly fewer NFT (hippocampus), NP (cortex, especially the superficial layers), and less %NP (cortex, hippocampus) (indicated by “*”) compared to P1, *i.e.* after 5 *versus* 12 years.

The ε4/4 APOE genotype was associated with excess AD pathology compared to ε2/3. Specifically, P3 had statistically significantly more NFT (cortex), NP (deep cortex), and higher %NP (cortex, hippocampus) than P1. The finding of a higher %NP for P3 (Fig. 1 c right), without elevated NP count, suggests larger plaques than for P1.

Eleven cortical areas

A detailed investigation of the cortex is shown in Fig. 2 a-c. There were 11 contributing cortical areas. Three measurements were made for the superficial and the deep layers. The median values

are shown as red squares and blue dots, respectively. The bars encompass the low and high values.

NFT were most common in the entorhinal cortex (superficial > deep layers) with similar median values for each subject (a). An excess of

Fig. 1 Summary of AD pathology in the three brains from subjects P1, P2 and P3. Note statistical significance: #-between the upper and lower cortical layers and *-between the individuals, a) neurofibrillary tangle density (N/cm²), b) neuritic plaques density (N/cm²), c) % of neuritic plaque occupancy.

Fig. 2 A detailed regional distribution of a) NFT, b) NP, and c) % of NP in three brains, P1,P2,P3. Red = upper layers, blue=lower layers. Red and blue lines represent the mean value across the all regions/ brain analyzed.

Fig. 3 Regional cortical pathology of three brains (a,c,e) and the interbrain differences (b,d,f) concerning neurofibrillary tangle density (N/cm²) (a,b), neuritic plaques density (N/cm²) (c,d), and % of neuritic plaque occupancy (e,f). The bars represent the mean values of the three individual measurements.

NFT in the superficial layers was not found for each area: the reverse was found for the temporal superior, frontal medial, supragranular and cingular areas. An excess of plaque (NP, %NP) in the superficial layers was a consistent finding for each region and subject (b-c). The associative cortex displayed rather wide interindividual variation. The primary cortex (precentral, postcentral, occipital I) contained relatively fewer neurofibrillary changes.

Five cortical regions

The 11 areas just described were then grouped into five regions/lobes to facilitate intersubject comparisons (Fig. 3 a-f): temporal (superior temporal, entorhinal) (red), parietal (postcentral, supramarginal) (yellow), frontal (prefrontal, medial, orbitofrontal, precentral) (green), limbic (anterior cingulate) (turquoise), occipital (primary and secondary visual areas) (royal blue). Solid colors denote superficial layers; horizontal white strips denote deeper layers.

Each subject demonstrated wide variation in NFT count: temporal lobe (highest) and occipital (lowest) (a). P2 had less regional variation compared to P1 and fewer temporal NFT (b). P3 had an excess of frontal and cingular NFT, especially for the deep cingular layers.

NP demonstrated lesser regional variation than was found for NFT. There was an excess of NP in the superficial layers for each region and subject (c-f). The highest levels were found for P3 in the superficial parietal cortex. The excess of parietal plaque for P3 (compared to P1) was clearly evident using %NP instead of NP (Fig. 3 f). P3 demonstrated an excess of NP (and %NP) in other regions: frontal, limbic, and occipital. Both P2 and P3 had less NP (and %NP) in the superficial layers of the temporal cortex compared to P1. Other regions, showed both more, and less NP (and %NP) for P2.

Hippocampus

Each subject demonstrated similar patterns of accumulation of hippocampal neurofibrillary changes (Fig. 4). P2 had fewer NFT in the CA1 (anterior + posterior), CA2, CA3 and CA4 areas

than P1. Excess NFT for P3 *versus* P1 was found only in the subiculum. NFT were not found in the dentatus. The redundant plaque for P1 *versus* P2 was found in CA1, subiculum and dentatus.

Other subcortical structures

There was evidence that dementia duration was associated with greater deposition of plaques and tangles, but no evidence for $\epsilon 4/4$ excesses. Note that P1 lacked amygdala information. NFT were found only in the NB Meynert and the amygdala (Fig. 5). For the NB Meynert, NFT count was lower in P2 than in P1, and equal for P3 and P1. For the amygdala, P2 had lower values than P3 and no information was available for P1.

The basal ganglia and cerebellum contained DP, not NP. Each structure showed distinctly less plaque for P2 than P1 (than P3 for the amygdala). Each structure also showed less (not more) for P3 than P1 (than P2 for the amygdala). Thus subcortical structures may continue to accumulate plaques and tangles during the clinical stages of AD. Age (not APOE genotype) also appeared to contribute to pathology in subcortical structures, *i.e.* P1 was older than P3.

Silver impregnation and APOE immunostaining

Bielschowsky staining of the front medial gyrus revealed an abundance of plaques for each subject, especially in the superficial layers (Fig. 6 a-c). Upon APOE immunostaining, dense staining of artery walls was found for each subject and each subject showed at least some staining in plaque-like structures. However, the amounts and distributions differed, suggesting more deposition of APOE associated with longer AD duration and with the occurrence of E4. P1 demonstrated few densely staining plaques, primarily in the superficial layers, with little immunoreactivity in the deep layers. P2 had almost no densely staining plaques in either superficial or deep layers. There was an abundance of densely staining plaques were found for P3 throughout all cortical layers (data not shown).

Fig. 4 Regional hippocampal pathology in the six subfields. The bars represent the mean values of the three individual measurements. a) neurofibrillary tangle density (N/cm²), b) neuritic plaques density (N/cm²), c) % of neuritic plaque occupancy.

Fig. 5 Regional subcortical pathology in P1, P2 and P3. Note that diffuse plaques only (DP) are found in basal ganglia and cerebellum. Other regions display neuritic plaques (NP). Note that amygdala region is missing in P1.

Fig. 6 Bielschowsky staining of the temporal medial gyrus for three subjects. Note that the cortical thickness in the P3 is decreased due to more severe atrophy. Bar = 500 µm.

Discussion

The regional brain AD pathology for three closely related men who carried the Swedish APP_{670, 671} mutation provides evidence that this variant of early-onset familial AD may represent a substantial acceleration of the sequenced neuronal disruption that is characteristic for late-onset familial/sporadic AD [41-46]. Each subject demonstrated quite similar regional brain distributions of neurofibrillary changes with the accumulation of NFT following the sequence of areas affected in late-onset AD pathogenesis: entorhinal cortex (outer > inner layers)

> hippocampus > other cortical regions. The CA1 and CA2 regions of the hippocampus had equal or more NFT compared to the entorhinal cortex for each subject. There were many NFT in cortical association areas and few in the primary areas. Each cortical region showed substantial NP deposits especially in superficial layers: temporal (highest) > parietal > frontal > limbic > occipital (lowest). NP were more dense in the neocortical areas than in the allocortical areas (hippocampus > entorhinal cortex). The observed patterns did not obviously deviate from those from late-onset AD in the Huddinge Brain Bank based on several hundred subjects [47].

By use of strict protocols concerning dissection, tissue processing and quantitative regional histology it was possible to demonstrate quantitative increases in neurofibrillary changes in the areas involved during earlier stages, within the highest Braaks' Stages V and VI with longer duration of dementia, *i.e.* for P1 compared to P2 [48]. Unexpectedly, longer duration was not associated with further accumulation of NFT in the entorhinal cortex, possibly due to a ceiling effect on the counting of NFT or resorption of early-deposited tangles. Longer duration was associated with more NFT in each hippocampal substructure and in the temporal areas and with elevated NP load in the entorhinal cortex (especially superficial), the hippocampus (especially the CA1, subiculum and dentatus), other subcortical structures, and temporal areas.

Quantitative increases and earlier onset were also found for $\epsilon 4/4$ compared to $\epsilon 2/3$ on this genetic background, consistent with findings for APP₇₁₇ and PS2 mutation carriers and also Down's syndrome patients. The excess frontal and limbic tangles and the clinical presentation of psychosis for P3 are intriguing, but could be consistent with relation between $\epsilon 4$ alleles, neurofibrillary pathology and region-specific involvement [49, 50, 51].

The 14 members of the kindred known to carry one or two $\epsilon 4$ alleles are too young to display AD symptoms and do not wish to be tested for the APP_{670/671} mutation. Indirectly, the study is consistent with, and extends, Bales *et al.* [52] who found that APOE is required for plaque deposition in homozygous APP_{V717P} transgenic mice: Abundant DP (only) was found without APOE. Here both genotypes supported plaque deposition: $\epsilon 2/3$ demonstrated large NP counts (and no DP) when one copy of APP_{670/671} was present. The absence of DP, excess of NP, possibly larger plaque size, and clear excess of APOE-immunostaining plaque for the $\epsilon 4/4$ carrier collectively imply an acceleration of the conversion of DP to NP plaque for $\epsilon 4$ along the lines suggested by Ishii *et al* [53].

The regional and layer neurofibrillary changes found in the study subjects can be used to infer which circuitry may be compromised in demented APP_{670, 671} carriers. It is suspected that the loss of neurofilament protein-rich pyramidal neurons

prone to develop NFT may herald the onset of AD dementia [54 for review]. These neurons are located in layers 3 (superficial) and 5 (deep) of the neocortex and, preferentially, layers 5 and 6 (deep) of the limbic cortex. Pyramidal neurons have long corticocortical, and corticosubcortical, projections. Their death disconnects diverse areas globally disintegrating thought processing.

While it is not entirely possible to classify corticocortical connections as feed-forward, feed-back, or lateral [55] due to the complexity of brain architecture [56], some generalizations are possible. Feed-forward projections often emerge from pyramidal neurons located in the superficial layers (mainly layer 3) and terminate preferentially in layer 4 of the targeting cortex. Feed-back projections mostly originate from pyramidal neurons in the deep layers (usually layer 5) which frequently terminate in layers 1 (superficial) and 6 (deep). It is likely that the latter connections are a requirement for learning processes, compromised in AD patients [57]. Lateral connections originate in the deep layers and terminate in all layers of the target region.

The disruption of feed-forward pyramidal neurons in demented APP_{670, 671} mutation carriers is suggested by the excess of NFT in the superficial layers of the temporal superior cortex, frontal (including medial, prepol, orbitofrontal areas), and the occipital (primary and secondary) cortex. Conversely, the excess of NFT in the deep layers of the parietal (supragranular) and limbic (anterior cingulate) cortex may indicate the early disruption of feedback neurons originating at these locations.

The 3-fold elevation of frontal and limbic NFTs for the study subject who carried the APOE $\epsilon 4/4$ genotype suggests that the $\epsilon 4$ allele may increase the vulnerability of specific corticocortical circuits, possibly accounting for the subject's emotional (and learning) problems. Feed-back circuits are implicated by the many tangles found in the deep layers of the cingulate cortex. In this regard, the frontal and limbic areas are heavily interconnected by reciprocal circuits (via both feedback and feedforward projections) [58]. The frontal dorsomedial area is interconnected with other associative areas in the inferior parietal and superior temporal regions [58, 59]. The parietal inferior cortex (which showed elevated plaque

load in its superficial layers for P3 and had 4-fold lower neuronal density compared to P1 counterpart – data not published), communicates in a reciprocal manner with the temporal superior, the frontal associative cortices, and receives rich input from the cingular cortex [58] and plays a pivotal role in circuits underlying cognition [59, 60]. Thus the increased parietal plaque load for subject P3 might have resulted from the NFT formation disrupting projections between the parietal cortex and temporal, frontal and limbic areas, by the mechanism previously proposed [61]. Moreover, animal studies clearly demonstrate that lack of APOE ultimately paradoxically leads to synaptic loss and neuronal degeneration impairing main morphological substrates compulsory for cognitive processing [62,63].

The 5-fold elevation in plaque load for P3 (*versus* P1) in the CA4 area of the hippocampus, may indicate a specific subcortical vulnerability of the CA4 neurons [64] related to the APOE ϵ 4 allele. Importantly, both NFT and NP were elevated in the subiculum for P3. This structure provides a gateway between the hippocampus and cortical structures that display heavy neuronal loss in late-onset AD [65]. Moreover, earlier appearance of neuropathological changes in the hippocampus was found in the brains of APOE ϵ 4 carriers compared with ϵ 2 or ϵ 3 carriers [31].

The excess of neuritic plaque in superficial *versus* deep, layers for each of 11 neocortical areas suggests that plaque is formed at the terminations of disrupted 1) associative and commissural feedback (and partially feedforward) projections, 2) lateral projections, and 3) projections from the brainstem. Such a scenario has been suggested elsewhere for late-onset AD [54]. Moreover, superficial layers are the place where rich apical dendritic branches of at least pyramidal neurons located in layer 2, 3 and layer 5, meet afferent inputs. Thus an excess NP is consistent with the abundance of neurofibrillary threads (representing dendritic and axonal pathology) in the superficial compared to deep layers for late-onset AD.

In summary, brain pathology in APP_{670, 671} mutation carriers with early onset AD demonstrated neurofibrillary changes similar to those found for late-onset familial/sporadic AD with evidence for further accumulation of NFT and

NP in early-affected regions in clinical AD. Thus both disorders might share a sequenced loss of pyramidal neurons that compromises integrative cognitive circuitry. The conjunction of elevated limbic and frontal NFT and psychosis in the APOE ϵ 4/4 carrier is intriguing. Plaque deposition may be accelerated by ϵ 4. Application of precise histologic methods, standardized across laboratories, would allow better definition of the morphologic substrate for AD in its various genetic contexts.

Acknowledgements

We thank Mrs I. Volkmann for skillful technical assistance. This work was supported by Gun and Bertil Stohne Foundation, Einer Belvén, National Institute on Aging Grant (P20-AG12852-02), Swedish Medical Research Council, Gamla Tjänarinnor, Loo och Hans Ostermans Fond, Alzheimer Fonden, Greta Lindénau-Hansells stipendium (N.B.).

References

1. **Cai X.D., Golde T.E., Younkin S.G.**, Release of excess amyloid β protein from a mutant amyloid β protein precursor, *Science*, **259**: 514-516, 1993
2. **Citron M., Oltersdorf T., Haass C., McConlogue L., Hung A.Y., Seubert P., Vigo-Pelfrey C., Lieberburg I., Selkoe D.J.**, Mutation of the β -amyloid precursor protein in familial Alzheimer's disease increases β -protein production, *Nature*, **360**: 672-674, 1992
3. **Mann D.M.A., Iwatsubo T., Ihara Y., Cairns N.J., Lantos P.L., Bogdanovic N., Lannfelt L., Winblad B., Maat-Schieman M.L., Rossor M.N.**, Predominant deposition of amyloid β 42(43) in plaques in cases of Alzheimer's disease and hereditary cerebral hemorrhage associated with mutations in the Amyloid Precursor Protein Gene, *Am. J. Pathol.*, **148**: 1257-1266, 1996
4. **Mullan M., Crawford F., Axelman K., Houlden H., Lilius L., Winblad B., Lannfelt L.**, A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of β -amyloid, *Nature Genet.*, **1**: 345-347, 1992
5. **Lannfelt L., Bogdanovic N., Appeltgren H., Axelman K., Lilius L., Hansson G., Schenk D., Hardy J., Winblad B.**, Amyloid precursor protein mutation causes Alzheimer's disease in a Swedish family, *Neurosci. Lett.*, **168**: 254-256, 1994

6. **Axelman K, Basun H, Winblad B, Lannfelt L**, A large Swedish family with Alzheimer's disease with a codon 670/671 amyloid precursor protein mutation: A clinical and genealogical investigation, *Arch. Neurol.*, **51**: 1193-1197, 1994
7. **Lannfelt L, Basun H, Wahlund L.O., Rowe B.A., Wagner S.L.**, Decreased α -secretase-cleaved amyloid precursor protein as a diagnostic marker for Alzheimer's disease, *Nature Med.*, **1**: 829-832, 1995
8. **Rogers J., Morrison J.H.**, Quantitative morphology and regional and laminar distributions of senile plaques in Alzheimer's disease, *J. Neurosci.*, **5**: 2801-2808, 1985
9. **Braak H., Braak E.**, Neuropathological staging of Alzheimer-related neurofibrillary changes, *Acta Neuropath.*, **82**: 239-259, 1991
10. **Morrison J.H.**, Differential vulnerability, connectivity and cell typology, *Neurobiol Aging*, **14**: 51-54, 1993
11. **DeLacoste M.C., White C.L.**, The role of cortical connectivity in Alzheimer's disease pathogenesis: a review and model system, *Neurobiol. Aging.*, **14**: 1-16, 1993
12. **Strittmatter W.J., Saunders A.M., Schmechel D, Pericak-Vance M, Enghild J, Salvesen G.S., Roses A.D.** Apolipoprotein E: high-avidity binding to β -amyloid and increased frequency of type ϵ 4 allele in late-onset familial Alzheimer disease, *Proc. Natl. Acad. Sci.*, **90**: 1977-1981, 1993
13. **Rebeck GW, Reiter JS, Strickland DK, Hyman BT.** Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions, *Neuron.*, **11**: 575-580, 1993
14. **Sanan D.A., Weisgraber K.H., Russell S.J., Mahley R.W., Huang D., Saunders A., Schmechel D., Wisniewski T., Frangione B., Roses A.D., et al.** Apolipoprotein E associates with beta amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE 3, *J Clin Invest*, **94**: 860-869, 1994
15. **Wisniewski T., Castano E.M., Golabek A., Vogel T., Frangione B.**, Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro, *Am. J. Pathol.*, **145**: 1030-1035, 1994
16. **Ma J., Yee A., Brewer H.J. Jr., Das S., Potter H., -** Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments, *Nature*, **372**: 92-94, 1994
17. **Brooks W.S., Martins R.N., Devoecht J., Nicholson G.A., Schofield P.R., Kwok J.B.J., Fisher C., Yeung L.U., Vanbroeckhoven C.** A mutation in codon 717 of the amyloid precursor protein gene in an Australian family with Alzheimers disease, *Neurosci. Lett.*, **199**: 183-186, 1995
18. **Alzheimer's Disease Collaborative Group**, Apolipoprotein E genotype and Alzheimer's disease, *Lancet*, **342**: 737-738, 1993
19. **St. George-Hyslop P., McLachlan D.C., Tsuda T., Rogaev E., Karlinsky H., Lippa C.F., Pollen D., Tsuda T.**, Alzheimer's disease and possible gene interaction, *Science*, **263**: 5146, 1994
20. **Hardy J., Crook R., Perry R., Raghovan R., Robeers G.**, ApoE genotype and Down syndrome, *Lancet*, **343**: 979-80, 1994
21. **Hyman B.T., West H.L., Rebeck G.W., Buldyrev S.V., Mantegna R.N., Ukleja M., Havlin S., Stanley H.E.**, Neuropathological changes in Down syndrome hippocampal formation: Effect of age and apolipoprotein E genotype, *Arch. Neurol.*, **52**: 373-378, 1995
22. **Levy-Lahad E., Lahad A., Wijsman E.M., Bird T.D., Schellenberg G.D.**, Apolipoprotein E genotypes and age of onset in early-onset familial Alzheimers disease, *Ann. Neurol.*, **38**: 678-680, 1995
23. **Van Broeckhoven C., Backhovens C.H., Cruts M., Martin J.J., Crook R., Houlden H., Hardy J.**, APOE genotype does not modulate age of onset in families with chromosome 14 encoded Alzheimer's disease, *Neurosci. Lett.*, **169**: 179-180, 1994
24. **Corder E.H., Saunders A.M., Strittmatter W.J., Schmechel D.E., Gaskell P.C., Small G.W., Roses A.D., Haines J.L., Pericak-Vance M.A.**, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families, *Science*, **261**: 921-923, 1993
25. **Basun H., Grut M., Winblad B., Lannfelt L., -** Apolipoprotein ϵ 4 allele and disease progression in patients with late-onset Alzheimer's disease, *Neurosci. Lett.*, **183**: 32-34, 1995
26. **Corder E.H., Saunders A.M., Strittmatter W.J., Schmechel D.E., Gaskell P.C. Jr., Rimmer J.B., Locke P.A., Conneally P.M., Schmechel K.E., Tanzi R.E., et al.** Apolipoprotein E, survival in Alzheimer's disease patients, and the competing risks of death and Alzheimer's disease, *Neurology*, **45**: 1323-1328, 1995
27. **Schmechel D.E., Saunders A.M., Strittmatter W.J., Crain B.J., Hulette C.M., Joo S.H., Pericak-Vance M.A., Goldgaber D., Roses A.D.**, Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease, *Proc. Natl. Acad. Sci.*, **90**: 9649-9653, 1993
28. **Saunders A.M., Strittmatter W.J., Schmechel, George-Hyslop P.H., Pericak-Vance M.A., Joo S.H., Rosi B.L., Gusella J.F., Crapper-MacLachlan D.R., Alberts M.J., et al.** Association of apolipoprotein E allele ϵ 4 with late-onset familial and sporadic Alzheimer's disease, *Neurology*, **43**: 1467-1472, 1993

29. **Strittmatter W.J., Weisgraber K.H., Huang D.Y., Dong L.M., Salvesen G.S., Pericak-Vance M., Schmechel D., Saunders A.M., Goldgaber D., Roses A.D.,** Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease, *Proc.Natl. Acad. Sci.*, **90**: 8098-9102, 1993
30. **Polvikoski T., Sulkava R., Haltia M., Kainulainen K., Vuorio A., Verkkoniemi A., Niinisto L., Halonen P., Kontula K.,** Apolipoprotein E, dementia, and cortical deposition of β -amyloid protein, *N. Engl. J. Med.*, **333**: 1242-1247, 1995
31. **Ohm T.G., Kirca M., Bohl J., Scharnagl H., Grob W., März W.,** Apolipoprotein E polymorphism influences not only cerebral senile plaque load but also Alzheimer-type neurofibrillary tangle formation, *Neurosci.*, **66**: 583-587, 1995
32. **Cedazo-Minguez A., Cowburn R.,** Apolipoprotein E: a major piece in the Alzheimer's disease puzzle, *J. Cell. Mol. Med.* **5**: 254-266, 2001
33. **Ghebremedhin E., Schultz C., Thal D.R., Rüb U., Ohm T.G., Braak E., Braak H.,** Gender and age modify the association between APOE and AD-related neuropathology, *Neurology* **56**: 1696-1701, 2001
34. **McKhann G., Drachmann D., Folstein M., Katzman R., Price D., Stadlan E.,** Clinical diagnosis of Alzheimer's disease, *Neurology*, **34**: 939-945, 1984
35. **Mirra S.S., Heyman A., McKeel D.,** The consortium to establish a registry of Alzheimer's disease CERAD. Part II standardization of the neuropathological assessment of Alzheimer's disease, *Neurology*, **41**: 479-486, 1991
36. **Bogdanovic N., Morris J.C.,** Diagnostic criteria for Alzheimer's disease in Multi-centre Brain Banking. In Cruz-Sanchez FF *et al.*, ed. Neuropathological diagnostic criteria for Brain Banking. Biomedical and Health Research, IOS Press, 1995, pp. 20-29
37. **Yamamoto T., Hirano A.,** A comparative study of modified Bielschowsky, Bodian and Thioflavin S stains on Alzheimer's neurofibrillary tangles, *Neuropath Appl Neurobiol*, **12**: 3-9, 1986
38. **Gundersen H.J.G., Bendtsen T.F., Korbo L., Marcussen N., Møller A., Nielsen K., Nyengaard J.B., Pakkenberg B., Sørensen F.B., Vesterby A., Mark W.,** Some new simple and efficient stereological methods and their use in pathological research and diagnosis, *APMIS*, **96**: 379-394, 1988
39. **Basun H., Corder E.H., Guo Z., Lannfelt L., Corder L.S., Manton K.G., Winblad B., Viitanen M.,** Apolipoprotein E polymorphism and stroke in a population sample aged 75 years or more, *Stroke*, **27**: 1310-1315, 1996
40. **Blennow K., Bogdanovic N., Alafuzoff I., Ekman R., Davidsson P.,** Synaptic pathology in Alzheimer's disease: relation to severity of dementia, but not to senile plaques, neurofibrillary tangles, or the APOE 4 allele, *J. Neural. Transm.*, **103**: 603-618, 1996
41. **Kemper T.,** Senile dementia: a focal disease in temporal pole. In Nandy K, ed. Senile dementia: a biomedical approach. New York: Elsevier, 1978 pp105-113
42. **Hyman BT, Van Hoesen GW, Damasio AR, Barnes CL.** Alzheimer's disease: cell specific pathology isolates the hippocampal formation. *Science*, **225**:1168-1170, 1984
43. **Hyman BT, Van Hoesen GW, Damasio AR.** Memory-related neuronal systems in Alzheimer's disease: anatomical study. *Neurology*, **40**:1721-1730, 1990
44. **Moossy J, Zubenko GS, Martinez J, Rao GR.** Bilateral symmetry of morphological lesions in Alzheimer's disease. *Arch Neurol*, **45**:251-254, 1988
45. **Arnold SE, Hyman BT, Flory J, Damasio A, Van Hoesen GW.** The topographic and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cerebral Cortex*, **1**:103-116, 1991
46. **Arriagada PV, Growden JH, Hedley-Whyte ET, Hyman BT.** Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer disease. *Neurology*, **42**:631-639, 1992
47. **Corder E.H., Woodbury M.A., Volkman I., Madsen D.K., Bogdanovic N., Winblad B.,** Density profiles of Alzheimer disease regional brain pathology for the Huddinge Brain Bank: pattern recognition emulates and expands upon Braak staging, *Exp Geront*, **35**: 851-64, 2000
48. **Ohm TG, Müller H., Braak H, Bohl J.** Close-meshed prevalence rates of different stages as a tool to uncover the rate of Alzheimer's disease-related neurofibrillary changes. *Neuroscience*, **64**:209-217, 1995
49. **Tekin S., Mega M.S., Masterman D.,M., Chow T., Garakin J., Vinters H.V., Cummings J.L.,** Orbitofrontal and anterior cingulate cortex neurofibrillary tangle burden is associated with agitation in Alzheimer disease, *Ann Neurol* **49**: 355-361, 2001
50. **Scarmeas N., Brandt J., Albert M., Devanand D.P., Marder K., Bell K., Ciappa A., Tycko B., Stern Y.,** Association between the APOE genotype and psychopathologic symptoms in Alzheimer's disease. *Neurology*, **58**: 1182-1188, 2002
51. **Mega M.S., Lee L., Dinov I.D., Mishkin F., Toga A.W., Cummings J.L.,** Cerebral correlates of psychotic symptoms in Alzheimer's disease, *J Neurol Neurosurg Psych* **69**: 167-171, 2000

52. **Bales K.R., Verina T., Dodel R.C., Du Y., Altstiel L., Bender M., Hyslop P., Johnstone E.M., Little S.P., Cummins D.J., Piccardo P., Ghetti B., Paul S.M.,** Lack of apolipoprotein E dramatically reduces amyloid β -peptide deposition, *Nature Gen.*, **17**:263-264, 1997
53. **Ishii K., Tamaoka A., Mizusawa H., Shoji S., Shoji S., Ohtake T., Fraser P.E., Takahashi H., Tsuji S., Gearing M., Mizutani T., Yamada S., Kato M., St George-Hyslop P.H., Mirra S.S., Mori H.,** $A\beta_{1-40}$ but not $A\beta_{1-42}$ levels in cortex correlate with apolipoprotein E $\epsilon 4$ allele dosage in sporadic Alzheimer's disease, *Brain Res.*, **748**: 250-252, 1997
54. **Hof P.R., Morisson J.H.,** Hippocampal and neocortical involvement in normal brain aging and dementia: morphological and neurochemical profile of the vulnerable circuits, *J. Am. Geriatric Soc.*, **44**: 857-864, 1996
55. **Felleman D.J., Van Essen D.C.,** Distributed hierarchical processing in the primate cerebral cortex, *Cerebral Cortex*, **1**: 1-47, 1991
56. **Barbas H.,** Pattern in laminar origin of corticocortical connections, *J. Comp. Neurol.*, **252**: 415-422, 1986
57. **Van Hoesen G.W.,** Cortical feedforward and cortical feedback neural systems in Alzheimer's disease. In: Hyman BT et al., eds. *Connections, Cognition and Alzheimer's disease*. Springer, 1997, pp. 17-31
58. **Hof P.R., Nimchinsky E.A., Morrison J.H.,** Neurochemical Phenotype of corticocortical connections in the macaque monkey: quantitative analysis of a subset of neurofilament protein-immunoreactive projection neurons in frontal, parietal, temporal and cingulate cortices, *J. Comp. Neurol.*, **362**:109-133, 1995
59. **Goldman-Rakic P.S.** Topography of cognition: Parallel distributed networks in primate association cortex, *Ann. Rev. Neurosci.*, **11**:137-56, 1988
60. **Selemon L.D., Goldman-Rakic P.S.,** Common cortical and subcortical targets of the dorsolateral prefrontal and posterior parietal cortices in the rhesus monkey: evidence for a distributed neural network subserving spatially guided behavior, *J. Neurosci.*, **8**:4049-68, 1988
61. **Trojanowski J.Q., Shin R.W., Schmidt M.L., Lee V.M.Y.,** Relation between plaques, tangles and dystrophic processes in Alzheimer's disease, *Neurobiol. Aging*, **16**:335-340, 1995
62. **Masliah E., Terry R.,** The role of synaptic pathology in the mechanisms of dementia of Alzheimer's disease, *Clin. Neurosci.*, **1**: 192-198, 1994
63. **Mattson M.P.,** Advances fuel Alzheimer's conundrum, *Nature Gen.*, **17**: 254-256, 1997
64. **West M.J., Coleman P.D., Flood D.G., Troncoso J.C.,** Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease, *Lancet*, **344**: 769-772, 1994
65. **Simic G., Kostovic I., Winblad B., Bogdanovic N.,** The volume and the number of neurons of the human hippocampal formation in normal ageing and Alzheimer's disease, *J. Comp. Neurol.*, **379**:482-494, 1997