TRUNCATED TAU PROTEIN AS A NEW MARKER FOR ALZHEIMER'S DISEASE

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Summary. – Molecular analysis of histological hallmarks (neurofibrillary tangles, neuritic plaques, neuropil threads and dystrophic neurites) of Alzheimer diseased brain tissues showed that these lesions contain paired helical filaments. Their major constituent is microtubule associated protein tau that is in an abnormally hyperphosphorylated and truncated state. These diseased forms of tau protein are unable to promote full microtubule assembly. Understanding of the molecular basis of the processes leading to the modifications of tau proteins and paired helical filament formation will form a firm step toward rational drug development and the cure of the Alzheimer's disease.

Key words: Alzheimer's disease; amyloid; apolipoprotein E; herpes simplex virus; neurofibrillary tangles; senile plaques; truncated tau protein

I. Introduction

Alzheimer's disease (AD) is the most common cause of late-life intellectual impairment in countries that have achieved life expectancies above 70 years. It accounts for about 60 % of the cases of dementia and its prevalence increases logarithmically with age (Multhaup *et al.*, 1993).

Demographic changes in the second half of the 20th century mean that as we approach the next millennium up to 40% of the population will be over the age of 60 by 2015 – 2020 (Mayer, 1993).

Due to gradual reduction in the numbers of neurones and synapses, clinical manifestations of AD include progressive impairments in short-term and - in later stages- in long term memory, language, behaviour and disorientation in time and space (McKhann *et al.*, 1986) usually leading to death in a severely debilitated, immobile state between four and twelve years after onset. The cost for looking after sufferers is enormous. For example the cost to American society for diagnosing and managing AD, primarily for custodial care, is currently estimated at more than $ 80 billion annually. No treatment that retards the progression of the disease is known (Selkoe, 1991). If scientific research does not produce discoveries to prevent or cure AD, the number of individuals affected can be expected to double by the year 2000 (14 million in the USA alone; Rossor,1993) and to quintuple by 2040 (Iqbal, 1991).

In contrast to other unsolved diseases of the human brain, classical pathology of AD continues to provide fertile basis that make it particularly amenable to studies of pathogenesis at the molecular level. Many molecular advances in research into AD have been made especially during last 10 years. Despite this progress, accumulation of the classical hallmarks, senile plaques and neurofibrillary tangles in the AD brains, observed 87 years ago by the Bavarian psychiatrist Alois Alzheimer in 1907, still remains the characteristic of AD. It is not known how the formation of these pathological structures occurs and how they contribute to the gradual reduction in the numbers of synapses and neurons associated with the cognitive decline typical of this disease. Understanding of the cellular and molecular biology of these processes is an essential prerequisite of the development of an early diagnostic assay and drugs capable of preventing steps leading to this neurodegenerative disease.

II. Hallmarks of AD

The intellectual decline in AD is accompanied by the progressive accumulation of insoluble fibrous material, both intracelullarly and extracellularly, in the brains of sufferers. Extracellular deposits are made of beta-amyloid protein (A4 protein) which is transformed into fibrils forming characteristic hallmark-senile plaque. The other hallmark of the disease which correlates with the degree of dementia is made of paired helical filaments (PHFs) found in neurofibrillary tangles, neuropil threads and as neuritic components of the neuritic plaques.

Senile and neuritic plaques

Senile plaques that occur within the cerebral cortex, the hippocampus, the amygdala and other parts of the brain are one of major hallmarks of AD with characteristic central deposits of extracellular amyloid fibrils (the core) surrounded by less densely packed amyloid (Fig. 1). Neuritic plaque is a more complex lesion of the cortical neuropil with amyloid core infiltrated and surrounded by dystrophic neurites (Mena *et al.*, 1991), fibrillary astrocytes (Wisniewski *et al.*, 1989) and activated microglia (McGeer *et al.*, 1987). Immuno-histochemical techniques revealed that senile plaques are often associated with various proteins (group of plaque associated proteins) including proteins of complement cascade (Eikelenboom and Stam *et al.*, 1982), cholinesterases (Mesulam *et al.*, 1987), alfa1-antichymotrypsin (Abraham *et al.*, 1988), heparan sulphate proteoglycan (Snow *et al.*, 1988), lysosomal proteases (Cataldo and Nixon, 1990). Products of both T- and B-cell compartments of the immune system have been identified in and around plaques including immunoglobulins (Ishii and Haga *et al.*, 1976) and thymus-dependent T-lymphocytes (Itagaki *et al.*, 1988) as well. The sequence of involvement of these factors in plaque formation and the time required to generate Alzheimer's disease type "mature" plaques are poorly understood. Neuritic plaques occur abundantly in two conditions: AD and trisomy 21. Innumerable non-compacted deposits of amyloid protein occur as "diffuse" or "preamyloid" plaques that contain very few or no surrounding dystrophic neurites or glia (Yamaguchi *et al.*, 1988). Senile plaques without neuritic component are often found during normal brain ageing. The fibrils of the plaque core are ultrastructurally distinct from PHF. Amyloid fibrils are extracellular, unpaired, and around 8 nm in diameter. They closely resemble the biochemically diverse amyloid filaments that accumulate extracellularly in non-neuronal tissues in a variety of unrelated systemic amyloidoses (Glen-

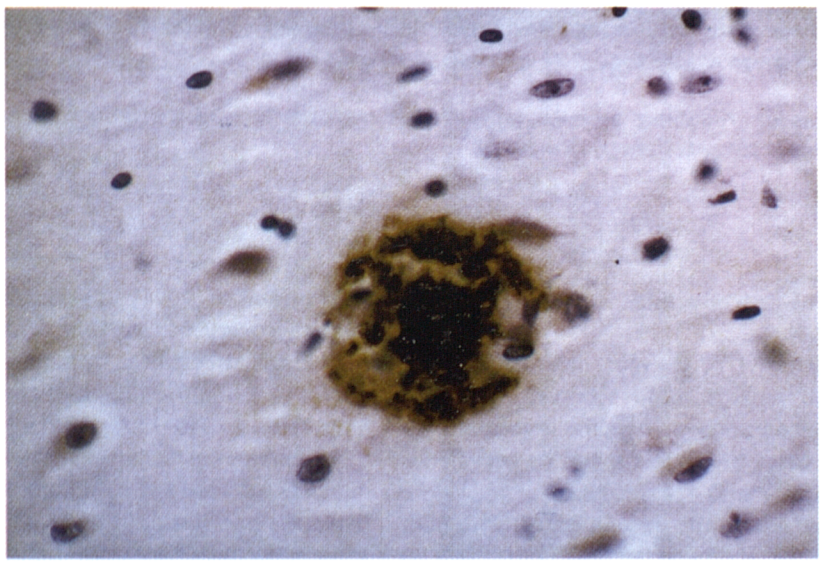

Fig. 1
Senile plaque
Classic senile plaque in the temporal cortex of AD brain tissue. Dense core of the plaque is clearly visible due to staining with MoAb specific to β amyloid/A4 protein.

ner, 1980). In addition to plaque cores, amyloid deposits occur in the walls of some or many cerebral and leptomeningeal blood vessels in AD.

It is important to stress that amyloidosis is a general term in pathology that designates disease in which deposits of 5 – 10 nm proteinaceous fibrils (amyloid) accumulate progressively in the extracellular spaces of tissues and their vasculature. Because the amyloid deposits in such disease invariably lead to local tissue injury rather than accumulate as inert by-products of the pathological process, it has long been assumed by some authors that this would also be the case in AD (Selkoe, 1991b).

Amyloid β protein (A4 amyloid protein)

Glenner and Wong (1984) were first to report the subunit composition of amyloid fibrils isolated from meningeal vessels. They sequenced a 4 K monomer up to residue 24 and found that this was a novel protein which was given name the amyloid β–protein (AβP or βA4 amyloid). Later, similar 4 K subunit protein with essentially identical amino acid composition was isolated from senile plaque cores of AD brain cortex (Masters *et al.*, 1985; Selkoe *et al.*, 1986). Complete cross-reaction of vascular and plaque amyloid was established with antibodies to AβP (Wong *et al.*, 1985; Selkoe *et al.*, 1986).

The intracellular processing of amyloid precursor protein (βAPP) and the pathways by which AβP (βA4) could either be generated or degraded have assumed an important position in research on AD. Sequence of βA4 protein is identical with residues 596 to 638/639 of βAPP695 (see below). The 42/43-amino acid βA4 protein comprises two domains: extracellular domain is represented by first 28 amino acid residues and transmembrane domain which is encoded by exon 17 (Kang 1987; Multhaup *et al.*, 1993). For 20 years it was believed by most of the researchers involved in study of AD that this fragment is central to the disease and that other hallmarks are secondary to amyloid. Abundance of senile plaques in AD brains heightened interest in the long standing and unresolved question of the origin of β-amyloid deposits in AD. The juxtaposition of the amyloid cores of classic plaques to degenerating neurites and activated glial cells has led to suggestions that the extracellular amyloid may arise from βAPP in altered neurones (Masters *et al.*, 1985), astrocytes (Siman *et al.*, 1989) or microglia (Wisniewski *et al.*, 1989). Strong support for the role of βA4 protein in AD came from studies of Down's syndrome, humans with trisomy 21. βAPP gene has been localised to the mid-portion of the long arm of the chromosome 21 (Selkoe, 1993). Studies of the brains of trisomic patients showed that diffuse AβP deposits could be detected in Down's subjects as early as in the mid-teens (Mann, 1989). At this age, few or no mature neuritic plaques, dystrophic glia, or neurofibrillary tangles are observed. Since the brain lesions of older trisomic 21 subjects are indistinguishable from those of AD it is possible according to Selkoe (1987; 1991) that similar deposition of Aβ4 in diffuse form precedes neuritic and glial alteration in AD as well. Since there does not exist any animal model for AD, the temporal progression of the lesions cannot be placed on firmer footing. Furthermore the whole amyloid hypothesis of the origin of AD will need more research before it will become really accepted across the field. Virtually all cells in the nervous system, including astrocytes, microglia, and endothelial cells as well as innumerable nonneuronal cells express βAPP. Furthermore, many diffuse AβP deposits in AD brain are now known to contain very few or no morphologically altered neurites (Yamaguchi *et al.*, 1988; Selkoe 1993). Furthermore it has been shown that the number of

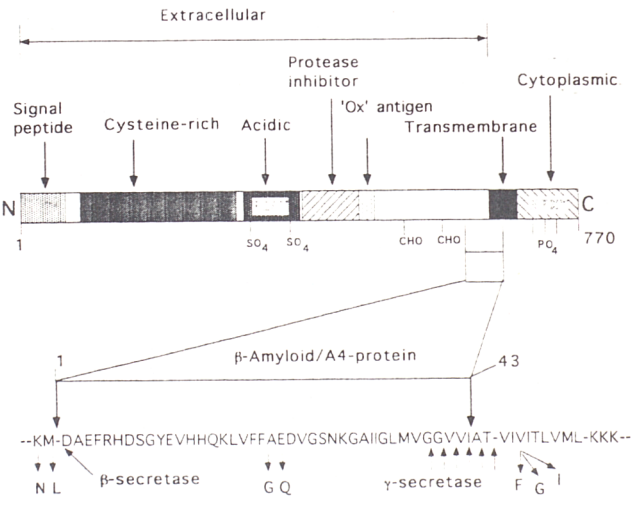

Fig. 2

β amyloid precursor protein (βAPP)

Structure of -amyloid/A4 protein and its precursor APP is shown. All numbering is according to the longest APP isoform (APP770). The A4 peptide region contains part of the transmembrane domain of APP and part of the extracellular domain. The β/A4 protein is a 39 – 42(43) amino acid long peptide which is derived from parts of exons 16 and 17 . Arrows within β/A4 indicate the site of constitutive enzymatic cleavage by APP secretase (Esch *et al.*, 1990). Later it was shown that there are more than one constitutive cleavage sites within APP protein (Selkoe, 1993). The two largest isoforms contain a Kunitz-type protease inhibitor domain (KPI). Mutations of APP are indicated by arrows. Swedish AD family has double mutation of Lys 670-Met 671 to Asn-Leu. Mutation Glu 693 to Gln results in HCHWA-D. Mutations at Val 717 to Phe, Ile or Gly are thought to result in AD (Ashall and Goate, 1994). Acidic domain in extracellular region contains PEST motif Pro (P), Glu (E), Ser (S) and Thr (T).This sequence is characteristic of a class of rapidly turning over proteins. This is consistent with the short half-life of APP. Tyrosine sulfation (SO4), N-glycosylation (CHO) and three phosphorylation sites (PO4), Thr 654, Ser 655, and Tyr 682 are shown (Weidemann *et al.*, 1989). Possible role in endosomal internalisation and degradation of amyloid precursor protein could be played by Asn, Pro, X and Tyr sequence (NPXY) that targets APP to clathrin coated pits and is located in the cytoplasmic domain.

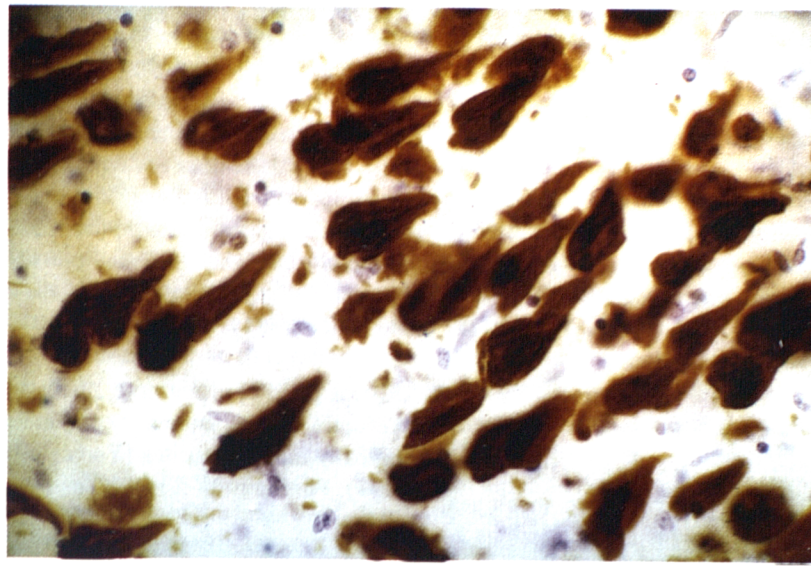

Fig. 3
Neurofibrillary tangles

Neurofibrillary tangles are classified on the basis of their staining into three categories: intracellular, transition and extracellular tangles (Bondareff *et al.*, 1990, 1991; Mena *et al.*, 1991). Figure shows massive incidence of neurofibrillary tangles stained with MoAb 423 in CA1 region of human hippocampus. Staining with this probe reveals that every single neurofibrillary tangle contains truncated tau at Glu 391 (Novak *et al.*, 1993*a*, 1994*b*). It has been established that the transformation of intracellular to extracellular tangles, in the course of neurofibrillary degeneration, is associated with the loss of fuzzy coat epitopes recognised by antibodies raised against the amino and carboxy-termini of tau protein, and the recognition of PHF core epitopes by antibodies which recognise epitopes in the vicinity of the repeated tubulin-binding region (Bondareff *et al.*, 1990).

senile plaques with neuritic component i.e. those with PHF correlate with the level of intellectual decline in AD but those without PHF i.e. "classic" amyloid plaques do not. Until now it had been assumed that the proteolytic release of βA4 from the transmembrane region of its large precursor protein was an aberrant event, requiring prior membrane injury. However, it has recently been shown that β-amyloid peptide is continuously secreted from healthy neural and non-neural cells in culture and circulates in human cerebrospinal fluid (CSF) and blood (Selkoe, 1993).

β *amyloid precursor protein*

The initial cloning of AP protein cDNA showed that AβP was a fragment of much larger 695 residue long precursor protein - amyloid precursor protein (APP) (Kang *et al.*, 1987), sometimes dubbed as preA4 (Fig. 2). Hence, the β-peptide is derived by proteolysis from this larger protein. The 39 – 42 residue AP region has 28 amino acids just outside of the cell membrane. Later it was shown that the single copy APP gene gives rise to a number of protein products. Alternative splicing leads to primary translation products of 365, 563, 695, 714, 751 and 770 amino acids, respectively (Kang *et al.*, 1987; DeSauvage and Octave, 1989; Golde *et al.*, 1990; Kang and Müller-Hill, 1990; Kitaguchi *et al.*, 1988; Ponte *et al.*, 1988; Tanzi *et al.*, 1988; Jacobsen *et al.*, 1991). These products represent the βAPP group of membrane-anchored polypeptides of 100 – 140 K that undergo N- and O-linked glycosylation, phosphorylation, sulfation and proteolytic cleavage and secretion of the large membranous region (Selkoe, 1993).

Two products of the βAPP group are 365 and 563 amino acid long and do not contain the βA4 (AβP) region which is encoded within exons 16 and 17 (Lemaire, *et al.*, 1989; Yoshikai *et al.*, 1990) and in order to distinguish them from APP which include AβP sequence they are dubbed as amyloid precursor-related proteins (APRP). The other proteins are APPs and as such typically glycosylated transmembrane proteins which span the lipid bilayer once (Dyrks *et al.*, 1988; Weidemann *et al.*, 1989). Novel leukocyte-specific APP forms (L-APP) which lack 18 amino acid residues close to the AβP region were recently detected (Konig *et al.*, 1992).

βAPP proteins are released from the cell surface by a secretase which is putative protease cleaving mature, N- plus O- glycosylated APP proteins at residue 687 (according to the longest isoform βAPP_{770} just outside the transmem-

brane region (i.e. at amino acid 16 of βA4) to release the soluble external domain (APPs). The secreted forms of the Kunitz-type protease inhibitor containing $APP_{770,751}$, L-$APP_{752,733}$ isoforms are identical to a previously described molecule, protease nexin II (PN-2) (Van Nostrand *et al.*, 1989; Goate *et al.*, 1991). PN-2 inhibits blood-clotting factor XIa, trypsin, chymotrypsin and the proteases termed epidermal growth factor-binding protein and g subunit of nerve growth factor (Smith *et al.*, 1990; Sinha *et al.*, 1990). The biological function of APP is not clear. APP may have a role in tissue repair in the brain; it is secreted by blood platelets. APP promotes cell-cell and cell-extracellular matrix adhesion. APP also promotes survival and growth of neurones *in vitro* (Ashall and Goate, 1994).

Neurofibrillary tangles

The second major neuropathological hallmark of AD are neurofibrillary changes in hippocampus and cerebral cortex as well as in some subcortical nuclei. Neurofibrillary changes are found in nerve cell bodies as neurofibrillary tangles (Fig. 3), in dendrites as neuropil threads and in the dystrophic neurites associated with senile plaques (Wischik *et al.*, 1990; Bondareff *et al.*, 1990, 1991; Novak *et al.*, 1993*a*, 1994*a*). It has been shown that the frequency of neurofibrillary changes is directly related to the degree of dementia in AD (Roth *et al.*, 1967; Blessed *et al.*, 1968; Wilcock and Esiri, 1982; McKee *et al.*, 1991; Arriagada *et al.*, 1992; Mukaetova-Ladinska *et al.*, 1993). Topographical distribution of these markers has been used for staging the progression of the disease (Braak and Braak, 1991). The main fibrous component of all neurofibrillary lesions described is PHF (Kidd,1963). It is significant that the same filaments are also found to accumulate in the abnormal neuritic processes of the neuritic plaques (Kidd, 1964; Wisniewski and Terry, 1970). Thus, a morphologically distinctive class of filament, the PHF occurs in the two main structural lesions of AD. Electron microscopic studies of the structure of PHF showed that the PHF appeared to consist of two filaments wound helically around one another, with a longitudinal spacing between crossovers of 65 – 80 nm and a width 27 – 34 nm at the widest part and 10 – 15 nm at the narrowest (Kidd, 1964; Wisniewski *et al.*, 1976).

The important question whether the intraneuronal degeneration that Alzheimer described is sufficient itself to derange mental functions attracted only a few scientist in the mid 80-ies. Then most of the researchers turned their attention to βA4 protein as a possible causative agent of AD mainly due to the work of Glenner and Wong (1984) and Masters *et al.* (1985) who isolated this peptide from brain amyloid accumulations in AD. Neurofibrillary changes were not considered as possible candidates for AD. In 1985 an international group of scientists under leadership and auspices of Nobel laureates Sir Aaron Klug, Director of Laboratory of Molecular Biology and its deputy Caesar Milstein was formed. Our goal was to study molecular biology of paired helical filaments as a perspective path leading to further clues to the etiology and cause of the disease. The scientific decision to study PHF in quite clear disagreement with world opinion turning its attention to βA4 peptide, was based on the facts that the tangle and neuritic plaque counts but not those of senile plaques correlate with clinical dementia (Roth *et al.*, 1967; Blessed *et al.*, 1968; Wilcock and Esiri, 1982; Mountjoy *et al.*, 1983; 1984). Furthemore they do correlate with the deficits in major neurotransmitter systems (Mountjoy *et al.*, 1984), and also with the decline in number of large cortical neurones which accompanies early-onset dementia (Mountjoy *et al.*, 1983). The evidence of tangle formation in the cells of origin of the cholinergic (Candy *et al.*, 1983), noradrenergic (Bondareff *et al.*, 1982) and serotoninergic pathways was seen as further support towards significance of neurofibrillary tangles and neurofibrillary pathology as a whole.

PHF consist of two structurally different parts: the core and outer fuzzy region

The strategy has been to develop methods for solubilizing the PHF or part of it, which is the nub of the biochemical problem, because of the well-known insolubility of the paired helical filaments (Selkoe *et al.*, 1982). This task was made all the more difficult because there is no functional or physiological assay for the protein components of the PHF. The only identification then possible was morphology of the PHFs at the electron microscope level. Since many conflicting claims have been made for candidate PHF proteins we have decided to use only well isolated individual filaments and not whole neurofibrillary tangles where other proteins may have been occluded. Antibodies selected on the basis of reactivity with tangles in histological sections (Anderton *et al.*, 1982; Dahl *et al.*, 1982; Ihara *et al.*, 1981; Nukina and Ihara, 1983; Mori *et al.*, 1987; Masters *et al.*, 1985; Wood *et al.*, 1986) are not reliable, because PHFs accumulate in the cytoplasm as densely packed aggregates that are likely to trap or become associated with many proteins that may have no bearing on assembly of the PHF. Even antibodies that can be shown to label isolated PHFs (Miller *et al.*, 1986; Perry *et al.*, 1985) do not necessarily establish the identity of the structural subunit of the PHF. We have found that paired helical filament of the neurofibrillary tangles characteristic of AD consist of two structurally distinct parts. An external fuzzy region can be removed by pronase treatment to leave a pronase-resistant morphologically intact core. Scanning transmission electron microscopy gives an estimate for the M_r per unit length as 79 K/nm before pronase

treatment and 65 K/nm after treatment (Wischik *et al.*, 1988*b*). The fuzzy region carries all the epitopes recognised by two different antibodies against microtubule-associated protein tau and anti-tau reactivity of the kind that has been reported (Wood *et al.*, 1986; Perry *et al.*, 1985) was abolished by this procedure. The M_r per unit length measurements imply that the three-domain structural subunit of the core has M_r about 100 K (Wischik *et al.*, 1988*a*,*b*).

The PHF analysis showed that removal of fuzzy outer domain does not affect the structural morphology of PHF but sharply changes the immunostaining profiles and it is no longer possible to prove if any epitope in the fuzzy coat is relevant to the inner structure of the PHF (Wischik *et al.*, 1988*a*). Therefore we have tried to develop such a marker, universal probe, which would allow us to identify core PHFs constituents and would work in immunoelectron microscopy, during immunohistological examinations, immunoblotting and ultimately as an probe for immunobiochemical assays for quantification of AD PHF-specific changes in the AD brain tissues (Novak *et al.*, 1989). For immunisation we have used pronase-treated PHFs to remove outer fuzzy coat which could induce interfering immune responses with our goal to construct hybridoma producing monoclonal antibody (MoAb) unequivocally recognising AD core PHF. After two years of work simultaneously developing unique techniques for solubilising the core PHF we have prepared hybridoma NOAL 6/66.423.2, which was derived by using the spleen of a C3H mouse hyperimmunized with the core PHF, producing MoAb 423 of isotype IgG2b. The hybridoma was selected on the basis of Immunogold decoration of the PHF core filament seen in the electron microscope (Novak *et al.*, 1989).

MoAb 423 is first and the only phosphorylation-independent core PHF-specific probe

To identify the components originating form the pronase-resistant core PHF we relied on MoAb 423 (Wischik, *et al.*, 1988*a*; Novak *et al.*, 1989), which labelled PHF cores strongly, and fuzzy PHFs only at higher antibody concentrations. Thus MoAb 423 identifies an epitope that is intimately associated with the pronase-resistant core of the PHF and is not directed against epitopes present in the fuzzy coat of the PHF (Wischik *et al.*, 1988*a*,*b*; Novak *et al.*, 1989). Using formic acid and sonication of the core PHF we have succeeded in releasing MoAb 423 immunoreactivity from the core PHF as an 12 K band which upon immunoblotting showed strong MoAb 423 staining. Sequencing of this band revealed an unknown sequence (Wischik *et al.*, 1988*a*). Part of the protein sequence Gln-Ile-Val-Tyr-Lys-Pro was used to design a mixture of oligonucleotides that served as a hybridisation probe to isolate a cDNA clone that led to its identification as human tau protein (Wischik *et al.*, 1988*a*; Goedert *et al.*, 1988). MoAb 423, which we have used to follow the 12 K peptide isolations and which decorates the PHF cores, does not cross-react with the fuzzy outer coat of PHF (Wischik *et al.*, 1988*b*). Therefore the 12 K fragment we have characterised as human tau protein that does not originate from the fuzzy outer material but is intimately associated with the protease – resistant core of the PHF (Wischik *et al.*, 1988*a*).

Tau protein is a major (if not the sole) component of the core PHF

All known tau isoforms (mouse, Lee *et al.*, 1988; Couchie *et al.*, 1992; human, Goedert *et al.*, 1988; 1989*a*; Andreadis *et al.*, 1992; cow, Himmler *et al.*, 1989; rat, Kosik *et al.*, 1989; Mori *et al.*, 1989; Kanai *et al.*, 1989; Goedert *et al.*, 1992) are produced from a single gene by alternative mRNA splicing. The human tau gene is located on the long arm of chromosome 17 at band position 17q21 (Neve *et al.*, 1986). As mentioned earlier, usage of 17mer oligonucleotide probes, derived from the protein sequence of

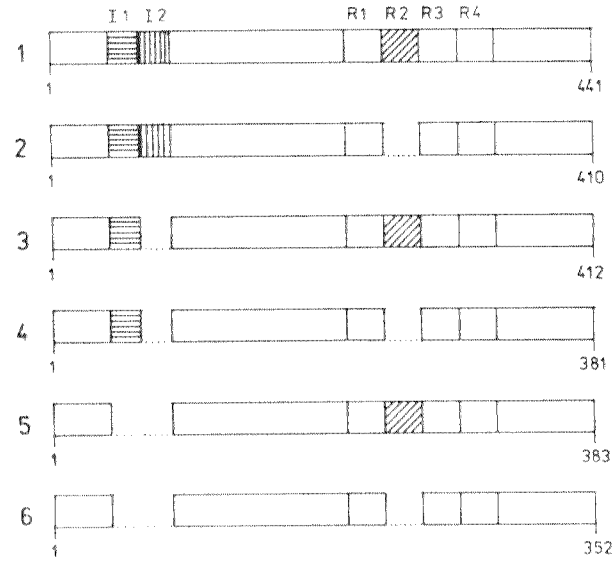

Fig. 4
Tau isoforms

Six tau isoforms are produced from a single gene through alternative mRNA splicing. They range from 352 to 441 amino acids and differ from each other by the presence or absence of inserts (I1 and I2) or one extra repeat (R2). Tau isoforms 1 and 2 contain 58 amino acid long inserts. Tau 3 and 4 contain N-terminal insert 29 amino acids long and isoforms 5 and 6 are without this insert. On the basis of the presence of an extra repeat (R2) in their microtubule binding region it is possible to divide tau isoforms into two groups, first (isoforms 1,3 and 5) with four repeats (R1,R2,R3,R4), and second (isoforms 2,4, and 6) having three repeats only (R1, R3, R4).

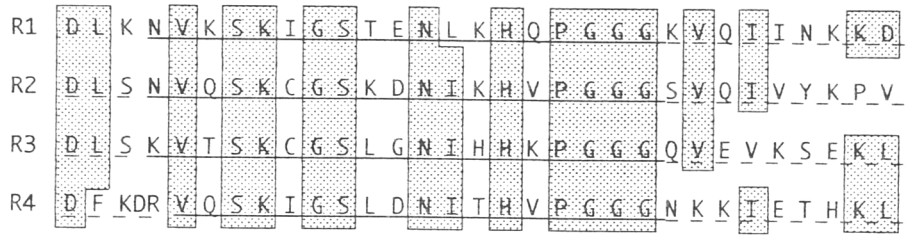

Fig. 5
Microtubule-binding domain
Four tandem repeats of human microtubule-associated protein tau constitute microtubule-binding domain and are located near the carboxy-terminus. Each repeat consists of the element 18 amino acids long (solid line) responsible for binding tau to tubulin, and linker region 13 amino acids long (dashed line). Most striking feature of microtubule binding region is PGGG motif which is repeated four times. Identical residues in the repeats are boxed and shadowed.

MoAb 423-positive core PHF fragment, allowed the isolation of a single hybridisation-positive clone from 650,000 clones of a cDNA library prepared from the frontal cortex of a patient who had died with AD. Obtained sequence showed high homology with mouse tau and was identified as a human microtubule-associated protein tau. Further screening cDNA libraries revealed that there are six various isoforms of the human tau protein (Fig. 4) that range from 352 to 441 amino acids in length and differ from each other by the presence or absence of two N-terminal inserts or three or four internal repeats (Goedert *et al.*, 1988, 1989). The most striking feature of the tau sequence is the presence of three or four tandem repeats of 31 or 32 amino acids (Fig. 5) located in the carboxy-terminal half, each containing a characteristic Pro-Gly-Gly-Gly motif (Goedert *et al.*, 1991). Experiments with synthetic peptides and with recombinant tau proteins show that the repeats constitute microtubule-binding domains (Ennulat *et al.*, 1989; Aizawa *et al.*, 1989) 18 amino acid long that are separated by apparently flexible linker sequences of 13 – 14 amino acids that do not bind (Butner and Kirschner, 1991). However, with recent findings showing that (a) linker region located between repeats 1 and 2 is unique microtubule binding site with more than twice the binding affinity of any individual repeat (Goode and Feinstein, 1994) and (b) that C-tail of tau encompasses significant microtubule assembly promoting region (Novak *et al.*, 1994*a*) it seems likely that the whole concept of repeat region will need detailed review. Transcripts encoding the various tau isoforms are expressed in a cell type-specific manner. *In situ* hybridisation has shown that tau protein mRNA occurs in nerve cells and that different neuronal cell types express different isoforms (Goedert *et al.*, 1989*a*). Transcripts encoding isoforms with three or four repeats are found in pyramidal cell bodies throughout all layers of the adult human cerebral cortex. No information is at present available about the cellular distribution of the various tau protein isoforms. Antibodies that recognise all tau isoforms label axons and only a small number of neuronal cell bodies and dendrites in adult brain from a variety of species (Binder *et al.*, 1985; Brion *et al.*, 1988; Migheli *et al.*, 1988).

The proof that at least part of tau protein forms a component of the paired helical filament core (Wischik *et al.*, 1988*a*,*b*) opened the way to understanding the mode of formation of paired helical filaments and thus ultimately in near future, the pathogenesis of AD. It has recently been shown that filaments similar but not identical can be assembled *in vitro* from non-phosphorylated fragments of tau, but not from the whole tau (Wille *et al.*, 1992; Crowther *et al.*, 1992). These results together with the molecular characterisation of abundant staining of AD tissues (Novak *et al.*, 1989, 1991, 1993*a*,*b*, 1994*a*,*b*; Bondareff *et al.*, 1990, 1991; Mena *et al.*, 1991, Caputo *et al.*, 1992) underlines the central role that tau plays in the neurofibrillary pathology of AD. Thus any knowledge about the physiology of microtubule-associated tau protein which could lead to an understanding of its role in pathology attains new significance. Moreover measurements of PHF tau concentrations showed 40 times higher levels in AD sufferers than in age matched controls (Harrington *et al.*, 1991; Wischik *et al.*, 1992; Mukaetova-Ladinska *et al.*, 1992, 1993). The most of the PHF tau in AD was found in grey matter where neurofibrillary tangles and dystrophic neurites are located in the somatodendritic compartment. The most affected regions were temporal and enthorhinal cortices, followed by parietal cortex, frontal cortex and hippocampus. The PHF content correlated strongly with the amount of dystrophic neurites but not with the diffuse βA4 plaques. Therefore it has been suggested that PHF content does not simply provide an alternative to histological counts of neurofibrillary tangles but measures the total PHF load within AD brain tissue (Harrington *et al.*, 1991; Mukaetova-Ladinska *et al.*, 1992, 1993). All assays

used were based on two MoAb 7.51 and MoAb 423, respectively. MoAb 7.51 is generic anti tau monoclonal antibody. It can measure amount of soluble tau and insoluble tau assembled into PHF (Novak *et al.*, 1991). MoAb 423 is specifically recognising truncated tau in neurofibrillary pathology of AD (Novak *et al.*, 1991, 1993*a*, 1994*a*,*b*).

Physiological functions of tau

The first tau function described was its potent capacity to promote polymerisation of tubulin *in vitro* (Weingarten *et al.*, 1975; Cleveland *et al.*, 1977*a*,*b*). It is broadly accepted that tau protein plays major role in tubulin polymerisation (Fig. 6) during neurite formation since it was found that levels of tau mRNA increase during neurite outgrowth. This increase correlates with an increase in the levels of polymerised tubulin. Microinjection of tau into fibroblasts

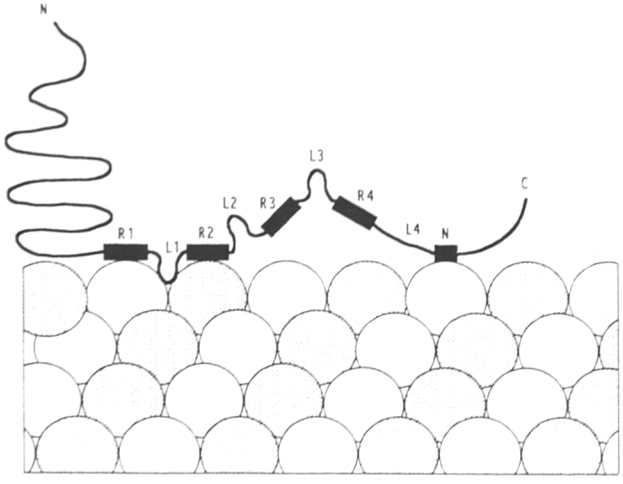

Fig. 6
Interaction between tau protein and microtubules
Diagram show an interaction between microtubules and tau protein. The framed area is expanded and shows a and tubulin subunits interacting with tau protein. Amino terminus (N) of tau is in the left upper corner. The locations of the four repeats are indicated by boxes (R1, R2, R3, R4). New potential "fifth" microtubule binding domain (N) is located in the C-tail (Novak *et al.*, 1994*a*). Linkers are represented by the hairpin structures separating the repeats. Recently, Goode and Feinstein (1994) identified a unique microtubule binding site, with more than twice the binding affinity of any individual repeat, in the first linker region (L1). Tau protein is flexible and is capable of multiple interactions with microtubules. It can stabilize microtubules without completely suppressing intrinsic polymer dynamics. The putative ability of tau protein to migrate even small distances on the microtubule lattice may explain how tau protein can function. Flexible binding domain is well suited to accommodate the curved and coiled geometries seen in intermediate stages of microtubule polymerisation (Butner and Kirschner, 1991). If these unusual properties of tau protein were modified by truncation or phosphorylation (Novak *et al.*, 1993*a*; Mandelkow and Mandelkow, 1993) in AD we might predict important consequences for basic neuronal processes e.g. transport and growth cones *in vivo*.

also leads to an increased resis tance of microtubules to depolymerising agents, suggesting a role for tau in stabilisation of assembled microtubules (Drubin *et al.*, 1985, 1986). With availability of tau cDNA clones many laboratories have transfected tau into eukaryotic cells that do not normally express tau protein. Kanai *et al.* (1992) engineered various tau constructs and studied the function of its individual regions on microtubule assembly and bundling of microtubules. Mutants lacking the proline rich region or the repeated domain did not bind to microtubules. Although all the other mutants could bind to and bundle microtubules, deletion in the N-terminal neutral region of the first half of the C-terminal tail caused a significant decrease in microtubule bundling, indicating the importance of these regions in microtubule bundling (Novak *et al.*, 1994*a*). Experiments with Sf9 insect cells infected with a baculovirus tau construct resulted in microtubule bundle formation and the appearing of neurite-like processes (Baas *et al.*, 1991). Anti-sense oligonucleotides prevented cerebellar nerve cells from developing axons (Caceres and Kosik, 1990). Another function of tau proteins was described by Hirokawa's group (Hirokawa *et al.*, 1988) namely the capacity of tau molecules to form cross-bridges of 18 nm length between adjacent microtubules. Under electron microscopy tau molecules resemble rods with a mean length of 35 nm and width of 3 – 5 nm. The microtubule binding domain alone measures 20 – 25 nm, thus leaving surprisingly only 10 – 15 nm for 3/4 of the length i.e. the whole N-terminal region plus the C-terminal tail. Butner and Kirschner (1991) showed that the binding affinity of tau is concentrated into four binding domains 18 amino acids long which are separated by linkers, 13 amino acids long. The same authors conclude their work , but suggesting that tau might adopt multiple conformations and possibly migrate on the surface of the microtubule with significant consequences for its function. It is quite possible that these properties of tau make it possible to take part in axonal transport. Molecular analysis of the C-terminal tail revealed that region around tau truncation is potent microtubule promoting region (Fig. 7) (Novak *et al.*, 1994*a*).

III. Pathology of tau protein in AD

There are two known pathological aberrations of tau protein in AD. Phosphorylation (Cleveland *et al.*, 1977*a*,*b*) and truncation of tau microtubule-associated protein (Novak *et al.*, 1993*a*).

Tau in AD is phosphorylated

Phosphorylation of tau has been described as early as 1977 (Cleveland *et al.*, 1977*a*,*b*). Phosphorylated tau was

weaker than non-phosphorylated tau in promoting capacity of microtubule assembly (Yamamoto *et al.*, 1983; Lindwall and Cole, 1984) thus showing that phosphorylation is after alternative splicing (which results in various tau isoforms) a second physiological regulatory mechanism modulating the affinity of tau proteins for microtubules. During foetal development tau is expressed as a single isoform (shortest) and is heavily phosphorylated. Phosphorylation disappears in the early postnatal period (14 days) and multiple isoforms start to appear. The main difference is that probably one or two residues are phosphorylated instead of 17 potential phosphorylation sites (Fig. 8) which could be phosphorylated *in vivo* during intrauterine development. It has been found that tau is readily phosphorylated *in vitro* by various kinases. Since the reappearance of the foetal pattern of

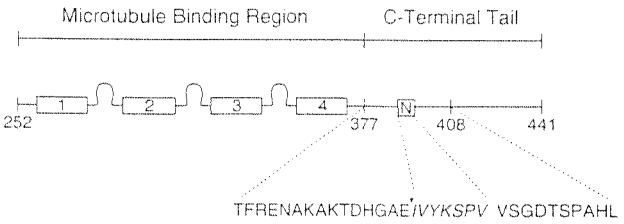

Fig. 7
Molecular analysis of C-terminal tail of microtubule
Sequence of C-terminal tail is identical in all six isoforms of human tau protein. Molecular reconstruction of tau truncation at Glu 391 revealed that downstream flanking region contains domain (N) with high microtubule assembly promoting capacity. We call this region "fifth" microtubule binding domain (Novak *et al.*, 1994*a*).

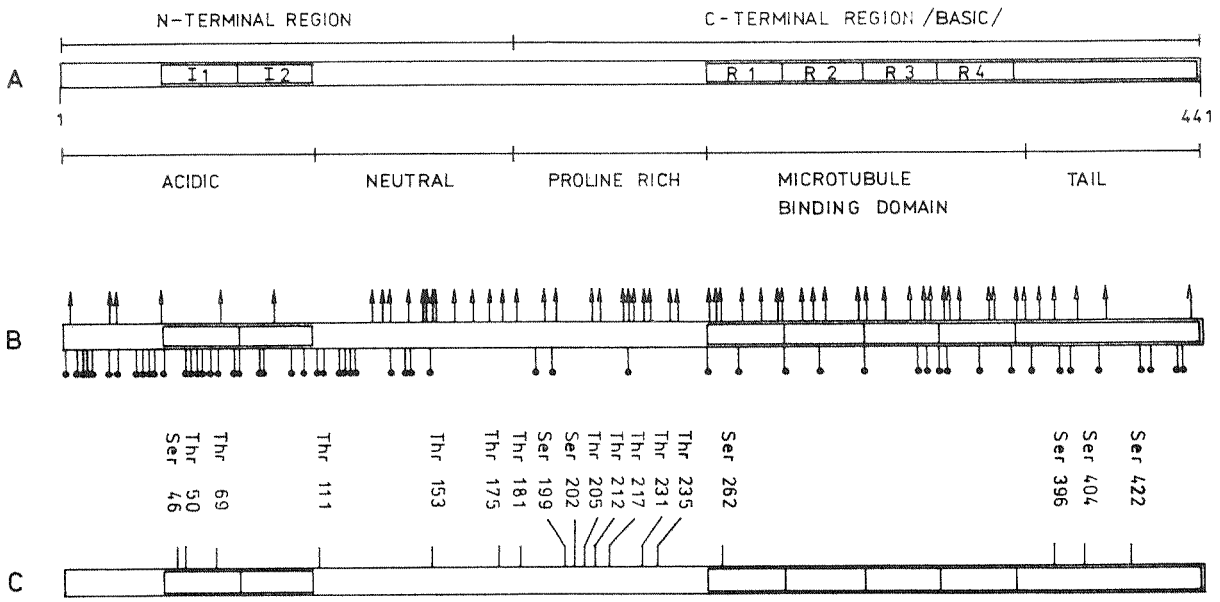

Fig. 8
Microtubule-associated protein tau
A. The bar represents the longest human tau isoform which has 441 residues. The whole molecule can be divided into two main regions, N-terminal and C-terminal region. There are two inserts (I1, I2) near the N-terminus. The C-terminal half harbours microtubule binding domain with four repeats (R1, R2, R3, R4). Furthermore it contains prolin-rich region and tail. Both regions play important role in microtubule assembly.
B. N-terminal half is acidic (dots) and C-terminal region is basic (arrows). Any alteration of charge could have devastating effect on tau function *in vitro*. Truncation of tau significantly alters the balance which in turn is reflected as 60% decrease in microtubule assembly promoting capacity (Novak *et al.*, 1993*b*).
C. Diagram showing the distribution of 17 potential phosphorylation sites of the Ser/Thr-Pro type and one (Ser 262) that does not contain previous motif and yet is under certain circumstances phosphorylated. Normal adult tau is phosphorylated at only a few of the 17 sites (probably two sites). AD tau is abnormally phosphorylated, i.e. many more sites are phosphorylated than in normal adult tau. Since phosphorylation-dependent antibodies recognise not only PHF-derived tau, but also tau from immature brain, some authors suggest that mechanisms underlying the phosphorylation of tau during development are thus reactivated in AD.

phosphorylation on tau isolated from AD brain tissues *in vitro* was recognised, phosphorylation attained major significance. *In vitro* microtubule-associated protein tau can be phosphorylated by multifunctional Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase) (Yamamoto *et al.*, 1983) This kinase phosphorylates a single serine residue

(Ser 416) at the C-terminal tail of the tau molecule (Steiner *et al.*, 1990). cAMP-dependent protein kinase, casein kinase I (Pierre and Nunez, 1983); protein kinase C (Hoshi *et al.*, 1987) phosphorylates Ser 262, 293, 324 and 356 (Correas *et al.*, 1992), casein kinase II (Steiner *et al.*, 1990), v-fms kinase (Kim *et al.*, 1991) proline-directed serine/threonine protein kinase (Correas *et al.*, 1992) cdc2 kinase (Drewes *et al.*, 1992), glycogen synthase kinase-3 (Hanger *et al.*, 1992) and mitogen-activated protein kinase (Drewes *et al.*, 1992). This kinase phosphorylates most of the 17 serine-proline and threonine-proline sites (Drewes *et al.*, 1992; Gustke *et al.*, 1992) and is capable of phosphorylating tau *in vitro* too. It is hoped that knowledge of the sites and the enzymes causing the disease will lead to a cellular model of AD which will allow a more detailed study of the disease (Mandelkow and Mandelkow, 1993) .

Truncated forms of tau AD form protease-resistant tauons

The AD PHF after digestion with pronase retains its characteristic morphological features. We term this the protease-resistant core PHF (Wischik *et al.*, 1988*a*,*b*). From this core we have released a 12 K tau fragment as an essentially pure preparation. Detailed sequence of this fragment revealed six distinct N-termini derived from the repeat region of 3- and 4- repeat tau isoforms. However, the 4-repeat isoforms released from the core lack either the first or the last repeat. The pronase-protected region of tau within PHF core is therefore restricted to three repeats, regardless of isoform. The alignment of cleavage sites at homologous positions within tandem repeats after protease treatment indicates that the tau-core association is precisely constrained by the tandem repeat structure of the tau molecule (Jakes *et al.*, 1991). Recombinant molecular reconstruction of this structure (Novak *et al.*, 1993*a*) revealed that it is made of 93/95 amino acid long tau fragments which we call "minimal protease resistant tau unit of the AD PHF". The set of these units forms protease-resistant filamentous structure which we have designated the tauons (see below). The C-terminus of the unit is a cleavage site at Glu-391 specifically recognised by MoAb 423. Addition or removal of a single residue abolishes MoAb 423 immunoreactivity. With the help of synthetic peptides and chemically produced MoAb 423-positive fragments we have found that the sequence recognised by MoAb 423 is Gly-Ala-Glu with an absolute requirement for glutamic acid being the last residue at the C-terminus (Novak *et al.*, 1994*b*). Since MoAb 423 does not recognise full length tau (Novak *et al.*, 1991) despite having internal GAE sequence and molecular reconstruction showed (Novak *et al.*, 1994*b*) that addition or removal of a single residue at the C-terminus abolishes immunoreactivity this MoAb, a unique probe for the measurement of core PHF truncation of tau protein in AD (Novak *et al.*, 1989; Harrington *et al.*, 1991). Molecular reconstruction of MoAb 423-identified truncation of tau revealed that expressed GAE truncated tau is 55% weaker at promoting of microtubule assembly than longest isoform of human tau with four internal repeats (Novak *et al.*, 1994*a*). This finding shows that the truncation effectively disables the tau protein and makes it available for some form of assembly into PHFs that leads to major pathological changes in neuronal cells (Fig. 8; Fig. 9). Furthemore we have expressed a set of truncated tau molecules in eukaryotic cells confirming the *in vitro* results. None of the truncated forms was able to bind

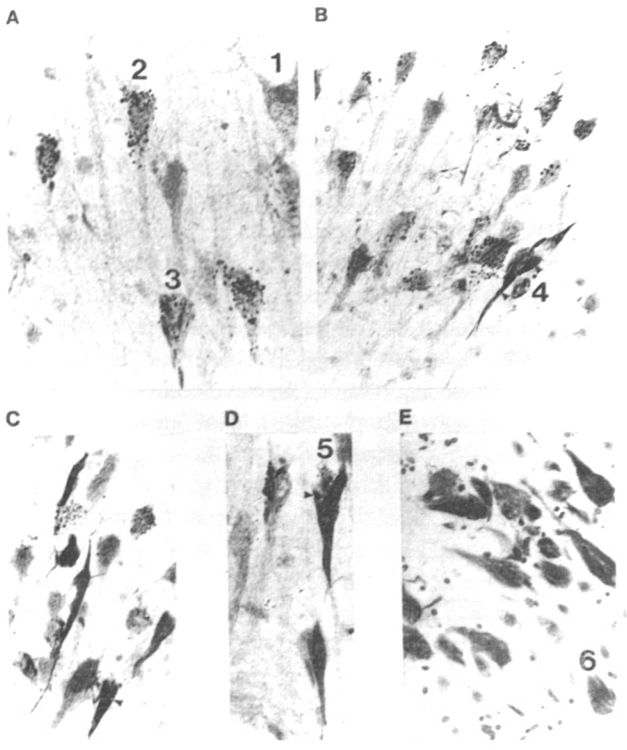

Fig. 9

Development of tau truncated protein deposits in AD brain tissue
Histological detection of C-terminal truncation of tau at Glu 391 in AD neurofibrillary pathology with MoAb 423. These can be classified according to 4 hypothetical stages. The earliest deposits of C-terminally truncated tau take the form of prominent granular inclusions within pyramidal cells vulnerable to neurofibrillary degeneration. These are clearly seen in A and B. At the next stage, tangle-like strands are seen to co-exist with granular deposits within the same cells (see arrowheads in B). At the third stage, tangle-like deposits occupy the whole cell (see arrows in C), and granular deposits are also occasionally seen to be present in the same cell (see arrow in D). Finally, as reported previously, swollen dispersed tangle-remnants in the extracellular space are labelled (E). Granular and fibrillar deposits within cells are optimally labelled by MoAb 423 following fixation in paraformaldehyde (A-D). Extracellular tangles are best visualised by MoAb 423 following fixation in methanol/acetic acid (E) (Novak *et al.*, 1993*b*, 1994*c*).
Magnification × 400.

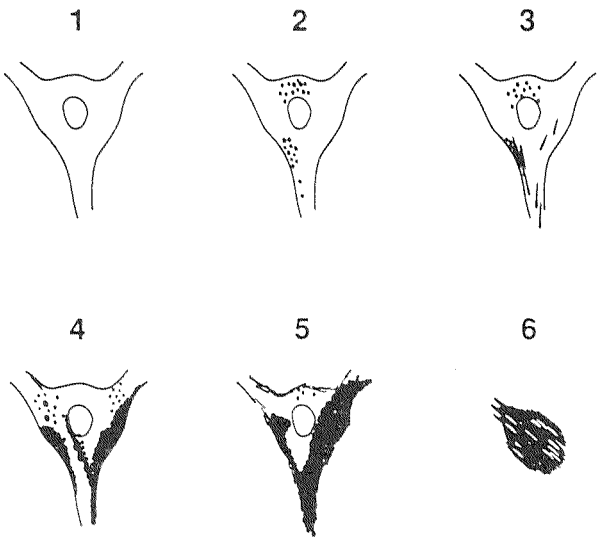

Fig. 10
Schematic representation of the depositions of MoAb 423-positive truncated tau protein
Schematic representation of the possible evolution of the deposition of MoAb 6.423 occurring in AD based on histological findings shown in previous Fig. 9.

to microtubules. It was possible to extract all disabled tau from the cells after permeabilisation thus confirming the *in vitro* results (Novak *et al.*, 1994*a*).

IV. Etiology of AD

The problem of the etiology of AD has not been solved. It is completely unknown. But in the past 10 years there have been significant extensions of our knowledge of the disease and advances in determining the molecular changes underlying the disorder. There is now convincing evidence that the dementia *per se* is caused by loss of neurons and synapses, particularly in the neocortex and hippocampus. The intracellular changes leading to accumulation of PHF within neurons have been outlined and partially defined. Modified forms of tau proteins have been identified and their pathological function characterised (Novak *et al.*, 1993*a*,*b*, 1994*a*; Mandelkow and Mandelkow, 1993). The molecular aspects of amyloid and its precursor protein have been defined (Ashall and Goate, 1994). For the first time, putative risk factors can be described in terms of pathogenic mechanisms. However, the ultimate answers about etiology of this devastating disease including its possible transmissibility will be provided in the near future only if substantial advances on the front of cellular and/or animal models will be achieved.

Risk factors for AD

The major risk factors for AD are age and genetic loci on chromosomes 14, 19 and 21 (Table 1). At least 10 pedigrees of early-onset, autosomal dominant familial AD exhibit linkage to missense mutations in amyloid precursor proteins (APPs) at codons 717 (numbering according to APP-770). The valine residue at this position is replaced by isoleucine, phenylalanine or glycine. These mutations occur within the transmembrane domain of APP, two amino acids downstream from the C-terminus of A4 protein (Goate *et al.*, 1991; Hardy *et al.*, 1991; Chartier-Harlin *et al.*, 1991).

Table 1. Classification of AD

Type	Chromosome	Gene/locus
1. Early-onset familial autosomal dominant	21	APP
2. Early-onset familial autosomal dominant	14	Unknown
3. Early-onset familial autosomal dominant (Volga-German)	Unknown	Unknown
4. Late-onset familial and sporadic association	19	Apo4
5. Other		

A double mutation of Lys 670-Met 671 to Asp-Leu immediately amino terminal to the β-peptide region occurs in a Swedish AD family. Mutation of Glu to Gln at position 22 of the β-peptide results in the hereditary cerebral hemorrhage with Dutch type amyloidosis (HCHWA-D). Another mutation, Ala to Gly at position 21 of the β-peptide (692 of APP), causes AD in some patients and HCHWA-D-like disease in others (Ashall and Goate, 1994). Moreover, recent studies have also identified a major early-onset locus on the distal part of chromosome 14 (14q24.3) (Schellenberg *et al.*, 1992; St.George-Hyslop *et al.*, 1992). Another candidate risk factor is exposure to increased levels of aluminium (Garruto, 1991). Using tau truncation-specific probe MoAb 423 (Novak *et al.*, 1989, 1991, 1993*a*,*b*,*c*) it was shown that dialysis patients, frequently exposed to high doses of aluminium, had increased levels of truncated tau

protein in the PHF-core fraction in white matter (Harrington *et al.*, 1994).

Apolipoprotein E (ApoE) type ε4 and pathological forms of tau protein: risk factor and perpetrator of the disease

Recently evidence has been provided that the ε4 allele of the principal transporter of cholesterol, ApoE (Fig. 11) , which is located on chromosome 19, is a susceptibility gene or risk factor in both sporadic and familial late-onset AD (Strittmatter *et al.*, 1993; Saunders *et al.*, 1993; Corder *et al.*, 1993). In an autopsy series of brains of late-onset AD patients, Strittmatter *et al.* (1993) found a strong association of ApoE ε4 allele with increased vascular and plaque βA deposits. Allen Roses , head of the group that discovered connection between ApoE and AD, presented results (Strittmatter *et al.*, 1994) suggesting that ApoE ε3 does bind to tau and through this binding this important protein is protected (Fig. 12). Therefore individuals with ApoE ε3 have an incidence of AD many times lower than people with ApoE ε4 . Rebeck *et al.* (1993) found that in sporadic AD 62% of patients possessed an ApoE ε4 allele, compared with 20% of controls. The ApoE ε4 isoform does not bind to tau and according to Strittmatter *et al.* (1994) this leaves tau protein unprotected and thus vulnerable to postranslational modifications which makes tau protein major perpetrator of the AD. This modifications could involve phosphorylation (Mandelkow and Mandelkow, 1993) or various truncations (Novak *et al.*, 1993*a,b*, 1994*a,b,c*) which could lead to assembly of PHFs and to neuronal cell death as seen in sections from AD brains stained with MoAb 423 (Novak *et al.*, 1989, 1991; Mena *et al.*, 1991).

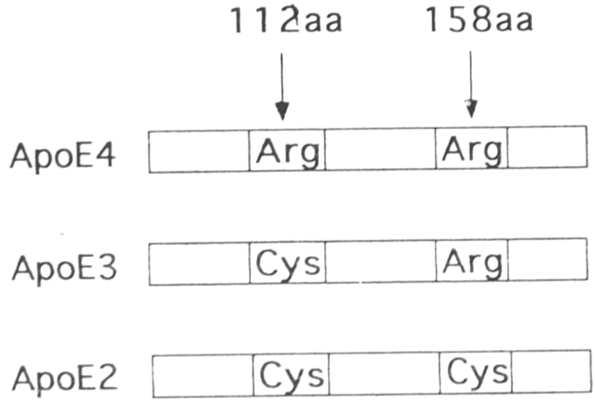

Fig. 11
Isoforms of apolipoprotein E (apoE)
Three common alleles of apoE encoding isoforms E2, E3 , and E4 have been identified (and several rare variants). The only differences among them are located to two residues at positions 112 and 158, respectively. ApoE is associated with AD neurofibrillary tangles and βA4 in senile plaques.

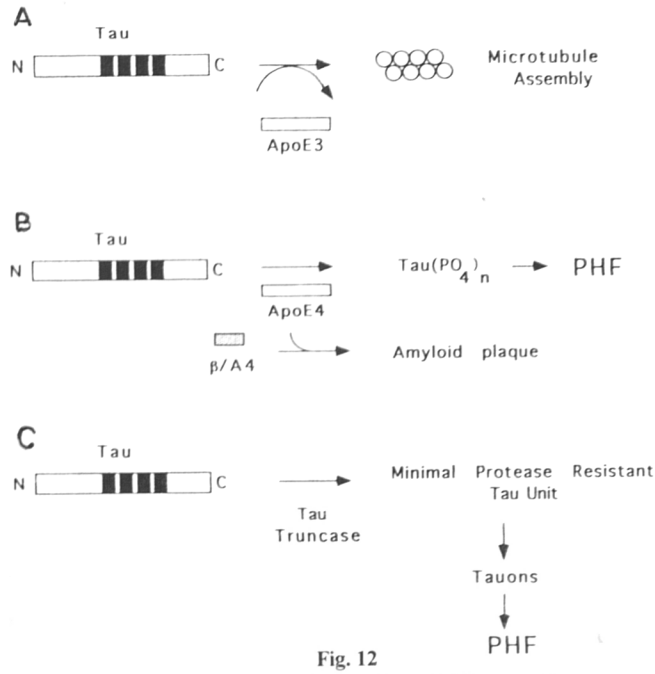

Fig. 12
Tau protein truncation, apoE and PHF assembly
Inheritance of ApoE4 is associated with increased risk and decreased age of onset of AD. ApoE4 does not directly cause the disease but is an important risk factor. Hypothesis "A" and "B" are based on recent studies which suggest that allelic variant-specific interactions of ApoE with tau may predispose an individual to an increased rate of neuronal injury, resulting in a decreased age of onset of AD (Strittmatter *et al.*, 1994).
A. ApoE3 binds to microtubule-associated protein tau thus protecting it from abnormal phosphorylation. Promotion of microtubule assembly and stabilisation of microtubules is not affected.
B. Binding of ApoE4 to βA4 may result in plaque formation. ApoE4 does not bind tau protein. This leaves tau unprotected and vulnerable to abnormal phosphorylation, self assembly and formation of PHFs.
C. Tau is truncated in AD. Truncation of tau by putative tau truncase produces 93/95 amino acids long minimal protease-resistant tau units which assemble into tauons (building blocks of PHF) and PHFs.

Transmissible "virus" dementias and AD

AD and spongiform encephalopaties/prion diseases share many common features. These chronic, progressive, sometimes familial diseases of the central nervous system are characterised by the presence of different types of amyloid and tau deposits in the brain.

Transmissible spongiform encephalopaties , Kuru, Creutzfeldt-Jakob disease (CJD) and Gerstmann-Straussler-Scheinker syndrome (GSS) belong to the group of virus-induced slow infections that have been described as subacute spongiform virus encephalopathies because of the strikingly similar histopathological lesions they induce

and the similarities of their infectious agents. To the same group belong scrapie, mink encephalopathy, chronic wasting disease with spongiform encephalopathy of captive mule de er and of captive elk, and bovine spongiform encephalopathy. Because of massive appearance of amyloid plaques structurally, ultrastructurally, and histochemically similar to the amyloid plaques of AD the Kuru disease was called "galloping senescence of the juvenile" and it was only after Merz *et al.* (1983, 1984) demonstrated on ultrastructural level that even those CJD patients without kuru plaques had amyloid fibrils, that were polymers of the same amyloid subunit, that it has been realised that all of these infections were transmissible cerebral amyloidoses (Diringer *et al.*, 1986; Gajdusek, 1985).

The past two decades have witnessed the collection of numerous experimental results which argue strongly that the scrapie agent is a novel pathogen. To distinguish the scrapie and CJD agents from viroids and viruses, the term "prion" was introduced (Prusiner, 1982). This consists of an abnormal cellular protein, designated the scrapie prion protein (PrP^{sc}). The single copy PrP^{sc} gene is located on chromosome 20. Transmissible spongiform encephalopathies (CJD, GSS, and familial fatal insomnia) are caused by specific mutations in this gene (Prusiner, 1991). Affected members of some CJD families have point mutations at codons 178 with change of aspartic acid to asparagine or 200 where glutamic acid changes to lysine (Goldfarb *et al.*, 1990, 1991; Hsiao *et al.*, 1991). Affected individual in GSS families have mutations at codon 102, 117, 198, or 217 (Goldgaber *et al.*, 1989; Hsiao *et al.*, 1989, 1991). One case has been described with GSS brain showing PrP^{sc} deposits as well as senile plaques and neurofibrillary tangles like in AD (Hsiao *et al.*, 1992; Ghetti *et al.*, 1989). The pathological hallmarks of transmissible encephalopathies are progressive vacuolization in the dendritic and axonal processes and cell bodies of neurons, astrogliosis, and in some combinations of strains of agent and host, neuritic plaques with amyloid cores which resemble those in AD (Fraser, 1976; Wisniewski *et al.*, 1975). While the nature of the amyloid differs in scrapie and AD (Prusiner *et al.*, 1983; Masters *et al.*, 1985*b*), the analogous pathological lesions of neuritic plaques and astrogliosis in scrapie and AD could imply convergent pathogenetic mechanisms. Transmissibility of encephalopathies is not general. For example Kuru does not transmit to sheep (Gibbs *et al.*, 1979). In monkeys with intracerebral inoculation of Kuru agent incubation periods have ranged from 1 to 12 years. This shows clearly that even the disease with well established transmissibility has a broad incubation period. In some cases prion diseases are neither spongiform (Collinge *et al.*, 1990) nor apparently transmissible (Brown *et al.*, 1991; Goldfarb *et al.*, 1992). The latest experiments with transmission of AD into monkeys showed (Baker *et al.*, 1993) that the brains of three marmosets monkeys injected intracerebrally 6 – 7 years earlier with brain tissue from a patient with early-onset AD contained moderate numbers of amyloid plaques with associated argyrophilic dystrophic neurites and cerebral amyloid angiopathy. The plaques and vascular amyloid stained positively with antibodies to β-amyloid protein showing that the amyloid is of "AD" origin. The brains of three age-matched control marmosets from the same colony did not show these neuropathological features neither did 12 further marmosets who had been injected several years ago with brain tissue which did not contain β-amyloid. These results show that cerebral β-amyloidosis of AD type can be induced by the introduction of exogenous amyloid β-protein.

"Classical" viruses and AD

Viruses have been implicated in several neurological diseases, for example, measles in subacute sclerosing panencephalitis (SSPE). Viral etiology has long been suspected for AD (Jamieson *et al.*, 1991). The agent which has been particularly highly implicated is herpes simplex virus type-1 (HSV1). Firstly, this is because the virus is known to have a predilection for residing in a latent state in neurons. Secondly, the virus is ubiquitous, and the prevalence appears to be at least 85% by the age 60 years in most populations studied (Nahmias *et al.*, 1990). Thirdly, the regions of the brain particularly affected in herpes encephalitis are those which show the main neuropathological features in AD, plaques and neurofibrillary tangles. Most previous attempts to detect HSV1 in AD or normal brain have proved unsuccessful (Middleton *et al.*, 1980; Pogo *et al.*, 1987). However, the techniques used were those of blot hybridisation, and the sensitivity in most studies was such that the virus would have been detected only if present at a level of one or more genomes per host cell genome and therefore this question was reexamined with a more powerful detection system, polymerase chain reaction method. Itzhaki *et al.* (1993) showed that the presence of HSV1 DNA sequences in human brain is strikingly dependent on age and on brain region. HSV1 DNA was detected in two thirds of the samples from aged normal individuals and in two thirds of those from the AD patients. The temporal cortex and the hippocampus were usually HSV-positive and occipital cortex has proved always to be HSV1-negative. The authors consider as significant that these HSV-positive and HSV-negative regions are precisely those most and least affected, respectively, in AD. The absence of HSV1 DNA sequence in brain from the middle-aged and from infants suggests that in general, entry of the virus into the CNS is restricted in younger individuals but that with increasing age, there is a decline in host resistance which permits access of the virus to the CNS. The fact that HSV DNA is present in a relatively high proportion of brain tissues from aged normal individu-

als does not preclude a role for the virus in AD. One can postulate that there are differences in virus or host characteristics between normal aged people and those suffering from AD which would lead to a greater extent or greater frequency of damage in the latter. An analogous situation occurs in the case of EBV. Nearly all humans have been infected with this virus but very few develop nasopharyngeal carcinoma or Burkitt's lymphoma-cancers which have been shown by strong, though only circumstantial, evidence, to be caused by the virus. As for the fact that not all brain specimens from AD patients are HSV1-positive, this could simply reflect the fact that the AD tissues have been taken post-mortem for these types of studies and that it is at least 4 – 10 years from clinically recognisable symptoms which could be a further 5 – 10 years from the real onset of disease when the virus could play decisive role. To answer this important question, early diagnostic assays for AD well before first clinical symptoms are recognisable and/or animal or cellular models for AD are needed. None of these is yet available.

Animal and cellular models for AD. Prions and tauons

So far no cellular or animal model for AD exists. All attempts to build such a models by well established groups in the field have ended in failure. The analogy that without animal model, it will not be possible provide satisfactory and conclusive answer about etiology of AD can be drawn from prion diseases. For example, although the unusual properties of the particle causing scrapie were first appreciated in the 1940s (Gordon, 1946), it was not until a mouse bioassay was developed by Chandler (1961) that sufficient data were obtained to suggest that the scrapie agent might not be a virus, bacterium, fungus, or parasite (Alper *et al.*, 1966; Prusiner *et al.*, 1979). The problem with animal models for AD is that no equivalent of the disease in animals including non-human primates have been found yet. Therefore it is our main scientific goal in the future to develop animal and cellular model(s) for AD which would enable us to address many relevant problems which we encountered during our research into the disease. For example it is essential to explore the possibility that minimal protease-resistant units of the AD pronase-resistant core being self-replicating units "tauons" similar to prions isolated from transmissible encephalopathies, and thus basic building blocks of intraneuronal PHFs that ultimately destroy the neurons in AD (Novak *et al.*, 1993, 1994*a*). PrP null mice do not exhibit a phenotype (Hsiao *et al.*, 1990) but transgenic mice with the 102 mutation develop disease (Bueler *et al.*, 1992). A feature of prion diseases which is always present is the synthesis and accumulation of protease-resistant PrP^{SC}, the principal component of the PrP amyloid plaques that occur *in vivo* (Kitamoto *et al.*, 1986). Partial proteolysis of PrP^{SC} (33 – 35 K) generates PrP 27 – 30, which forms amyloid rods *in vitro* (McKinley *et al.*, 1991) and it is believed that PrP^{SC} is derived from PrP^{C} via a posttranslational conformational modification (Prusiner *et al.*, 1990). PrP^{SC} (209 amino acids long) is defined as a form of PrP^{C} that readily forms protease-resistant aggregates after treatment with detergents. PrP^{SC} is partially resistant to proteinase K and yields PrP 27 – 30 (142 amino acids long with frayed N-terminus ranging from residues 73 to 90) after digestion (Weissmann, 1994). Tauons are minimal protease-resistant tau units of 12 K which form the core of the AD PHF (Novak *et al.*, 1993*a*,*b*, 1994*a*). They are unlike prions totally resistant to proteinase K and they can be chemically released from PHF. Furthermore they are resistant to the broad spectrum protease-pronase. Tauons in PHF undergo self assembly via unknown mechanism(s) within pyramidal cells of the brain which ultimately completely destroys them. The proposed candidate posttranslational mechanisms that change normal tau into pathological one are truncation (Novak *et al.*, 1993*a*,*b*, 1994*a*) and/ or phosphorylation (Mandelkow and Mandelkow, 1993). With advance in cellular or animal models for AD it will be possible to answer question about their putative "infectivity". The fact that tauons are not present in prion diseases suggests that new unknown self-replicating mechanisms could be involved in formation of PHFs.

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