

Chapter 39

Alzheimer's disease: a true tauopathy fueled by amyloid precursor protein dysfunction

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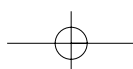
INTRODUCTION

Plaques or tangles?

We know from the pioneer work of Alois Alzheimer¹ that Alzheimer's disease (AD) is characterized by two types of lesion: one is extracellular, corresponding to spherical deposits, and the other is intraneuronal and composed of a fibrillar material named neurofibrillary tangles. These two types of lesion in high number in neocortical regions are the consensus criteria for the neuropathological diagnosis.² Biochemical analyses starting in 1984 demonstrated that extracellular amyloid plaques are made of a peptide named amyloid- β ($A\beta$), of 40–43 amino acid residues.³ The intraneuronal filamentous lesions, formed by bundles of paired helical filaments, result from the assembly of tau proteins.⁴ From the beginning of Alzheimer's research history, researchers have attempted to determine the cause of Alzheimer pathology: plaques or tangles? Or, in molecular terms, $A\beta$ deposition or tau aggregation?^{5–9}

Amyloid precursor protein dysmetabolism is central in Alzheimer's disease etiology

At the present time, the initiating cause of neurodegeneration in Alzheimer's disease is still a matter of debate. There is, however, no doubt that amyloid precursor protein (APP) dysfunction is a key event in AD, because mutations altering APP metabolism, such as mutations in APP and presenilin, invariably provoke AD with an early onset. Both familial AD, which is extremely rare, and sporadic AD, have the same distribution and number of amyloid plaques, demonstrating that APP metabolism is central in AD. For sporadic AD, which represents more than 99% of all cases, a number of hypotheses to explain neurodegeneration have been proposed, including the extracellular or intracellular neurotoxicity of $A\beta$, or a loss of function of APP. In the absence of any genetic mutation, physiopathological processes leading to amyloid deposition and neurodegeneration in sporadic AD remain poorly understood and poorly investigated. The fact is that scientific



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investment in AD has been inversely proportional to the most represented form of AD and hence, the amyloid cascade hypothesis reflects the major trend of research. This theory relies on aggregated A β as the neurotoxic substance provoking the disease.¹⁰ According to this theory, removing A β should cure the disease.¹¹

Tau pathology is well correlated with cognitive impairment

In parallel, neurofibrillary degeneration, i.e. tau pathology, has been described in the amyloid cascade hypothesis as a late and secondary event, in perfect disagreement with most neuropathological studies. Indeed, all these studies emphasize that neurofibrillary degeneration is also instrumental in the disease.^{9,12-17} Our biochemical spatiotemporal analysis of tau pathology in non-demented elderly patients from a prospective and multidisciplinary approach corroborates neuropathological findings.¹⁸ Indeed, neuropathological as well as biochemical approaches show that tau pathology of AD spreads progressively, invariably hierarchically, from the trans-entorhinal cortex to the whole neocortex, along corticocortical connections. The brain regions that are sequentially affected explain the successive types of cognitive impairment that characterize the disease: amnesia following the entorhinal and hippocampal degeneration; aphasia, apraxia and agnosia with the involvement of the neocortex. Of course, amyloid and tau pathology are present before the clinical symptoms, because neuronal plasticity is able to compensate at the first AD stages. Our studies have shown that tau pathology is already distributed in the hippocampal formation and the temporal cortex at the 'pre-clinical' stage of AD.⁸

The questions for this new century

Most of the researchers nowadays are convinced that APP dysfunction as well as tau pathology are important physiopathological factors. However, the precise relationship between these two very different degenerating processes remains a mystery. Understanding the spatiotemporal connection between APP and tau pathology could not only produce a better explanation of AD, but could also open new therapeutic perspectives. Moreover, it is also well established that clinical cognitive impairment is already a late event in the physiopathological development of AD. Therefore, it is essential to investigate both APP and tau pathologies in non-demented patients who have already developed the neuropathological stigmata of the disease. For that purpose, our strategy has been to study the distribution of APP and tau abnormal catabolic products in different brain regions of numerous patients, at different ages and with different cognitive skills or impairments, from perfect memory to dementia, and followed prospectively with a multidisciplinary approach. The aim was to study all possible cases from non-affected to fully affected patients. If the disease spreads along a precise path, we should be able to align all the different pieces of the puzzle along this path.

RELATIONSHIP BETWEEN DEMENTIA, TAU AND A β PATHOLOGY

Tau pathology spreading in cortical areas is invariable and hierarchical

The prospective and multidisciplinary study of more than 200 cases, including 70 non-demented patients, showed that tau pathology always extends along ten stages, corresponding to ten brain areas that are successively affected. Paired

helical filaments (PHF)-tau pathology, visualized as a triplet of abnormal tau proteins, was systematically found to be present in variable amounts in the entorhinal and hippocampal regions of non-demented patients aged over 75 years. When tau pathology was found in other brain areas, it was always along a stereotyped, sequential, hierarchical pathway. The progression was categorized into ten stages according to the brain regions affected: trans-entorhinal cortex (S1), entorhinal cortex (S2),

hippocampus (S3), anterior temporal cortex (S4), inferior temporal cortex (S5), mid-temporal cortex (S6), polymodal association areas (prefrontal, parietal inferior, temporal superior) (S7), unimodal areas (S8), primary motor (S9a) or sensory (S9b, S9c) areas, and all neocortical areas (S10) (Figure 39.1, left). Up to stage 6, the disease could be asymptomatic. In all cases of our study, stage-7 individuals with two polymodal association areas affected by tau pathology were cognitively impaired.¹⁸

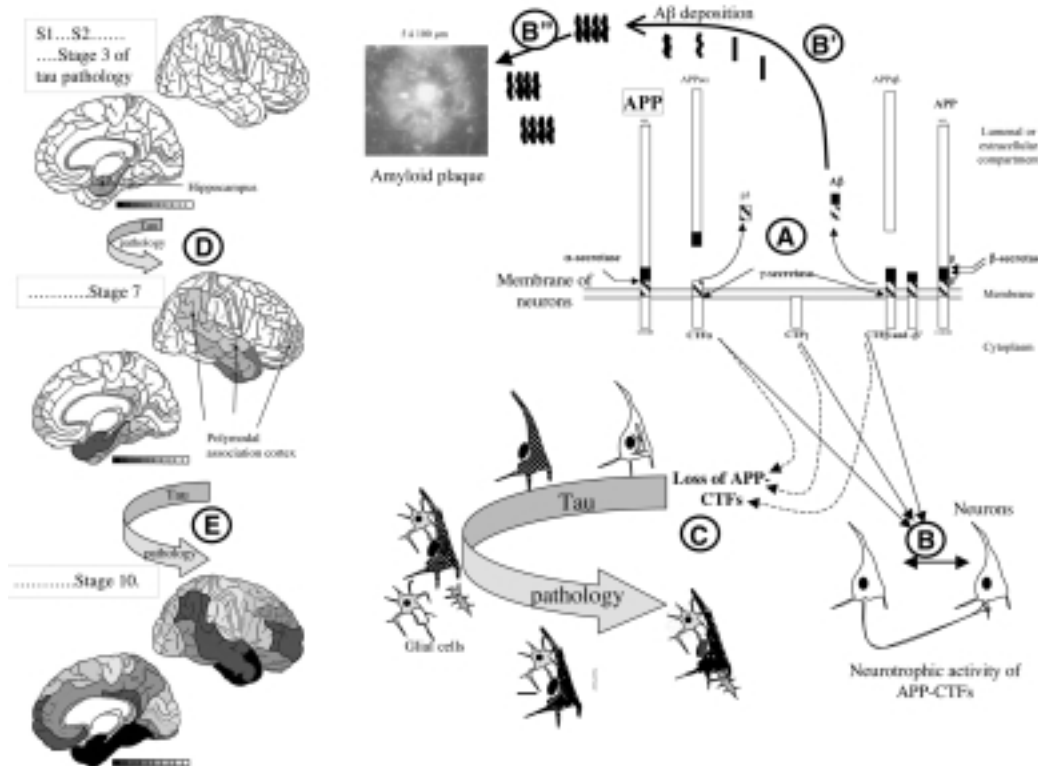


Figure 39.1 The possible synergy of amyloid precursor protein (APP)-tau pathologies in Alzheimer's disease. APP is a trans-membrane protein cleaved by different secretases, at the α , β , β' and γ sites (A). The cleavages release several APP carboxy-terminal fragments (APP-CTFs), which probably have a neurotrophic activity (B). Final secretase activities release, in parallel, A β peptide (B') that will aggregate into plaques (B''). A decrease of APP-CTFs is observed in Alzheimer's disease, which probably enhances tau pathology and neurodegeneration (C). The stimulated tau degenerating process progresses in brain areas, along corticocortical connections, from stages 1 to 3 (entorhinal then hippocampal formation) to stage 10 (all neocortical areas, via polymodal association areas (stage 7) (D and E)

Distribution of A β 42 and 40 species during the course of Alzheimer's disease

Insoluble A β 42 and 40 species were fully solubilized and quantified in the main neocortical areas, with a new procedure adapted to human brain tissue. The nature and quantities of A β species that aggregate were compared to the extent of tau pathology, as well as to cognitive impairment. In AD, there was a constellation of amyloid phenotypes, extending from cases with exclusively aggregated A β 42 to cases with, in addition, large quantities of insoluble A β 40 species. Nonetheless, insoluble A β 40 detection was often observed late in the amyloid deposition process (starting at stages 4–5). Moreover, we observed that there was no obvious spatial and temporal overlap in the distribution of these two insoluble A β species in cortical brain areas. The physical properties were also different. Formic acid-solubilized A β 40 aggregates were composed essentially of monomers and dimers, while solubilized A β 42 was essentially observed as dimers and multimers. More importantly, A β 42 aggregates were observed at the early stages of tau pathology, in non-demented patients, whereas the insoluble A β 40 pool was found at the last stages of AD, in demented patients.

All together, it was interesting to note that, during the progression of the disease, A β aggregates increased in quantity and heterogeneity, in close parallel with the extension of tau pathology. Unexpectedly, however, there was no spatial overlap between A β aggregation that was widespread and heterogeneously distributed in cortical areas, and tau pathology that was progressing sequentially, stereotypically and hierarchically. Therefore, these observations demonstrate that A β 42 aggregation, and not A β 40, is the marker that is close to Alzheimer etiology. It should be the main target for the early biological diagnosis of AD and modeling. Furthermore, the spatial mismatch between A β

and tau pathologies in cortical areas was obvious. First of all, the neocortical areas that first accumulate A β , or contain more A β , were not always the same. Generally, but not always, the occipital cortex was more prone to develop amyloid deposits. Remarkably, this region was the last to develop a tau pathology. In contrast, the hippocampal region, which is affected early by tau pathology, was not especially affected by amyloid deposition. These observations confirm the findings of Braak and Braak.¹⁴ Together, this A β /tau mismatch demonstrates that neurodegeneration is not a direct consequence of extracellular A β neurotoxicity (considering that toxicity is mediated through the cell body). Hence, there is a synergistic effect of APP dysfunction on the neuron-to-neuron propagation of tau pathology. This is demonstrated by the fact that tau pathology can be found in the hippocampal area without A β deposits. However, the extension of PHF-tau in the polymodal association areas was always found in the presence of A β deposits, as if these species, directly or indirectly, were necessary to stimulate the progression of tau pathology.¹⁹

Distribution of N-truncated A β species in Alzheimer's disease

During our quantification of A β 42 aggregated species in non-demented individuals, we noticed that the 4-kda band, corresponding to A β monomers, was extremely heterogeneous, as shown by immunological tools against the amino-terminal region of A β .¹⁹ This led us to analyze these species with a proteomic approach. We were more than surprised to observe that amino-truncated species represented more than 60% of all A β species, not only in full-blown AD, but also, and more unexpectedly, at the earliest stage of Alzheimer pathology. At this stage (non-demented patients



with diffuse A β deposits), A β oligomers consisted exclusively of A β 42 species, most of them being amino-truncated. Thus, our results strongly suggest that amino-truncated A β 42 species are instrumental in the amyloidosis process. Since vaccination has been proposed to remove amyloid deposits, the possible cause of AD, and because vaccination against the 'physiological' A β peptide provoked severe adverse events, a vaccine specifically targeting these pathological amino-truncated species of A β -42 are likely to be doubly beneficial, by inducing the production of specific antibodies against pathological A β products that are, in addition, involved in the early and basic mechanisms of amyloidosis in humans.²⁰ Moreover, the early implication of the amino-truncated A β 42 peptides in amyloidosis supports the usefulness of these species for an early, specific and accurate biological diagnosis of AD. Preliminary data show their specific presence, detected by mass spectra, in the cerebrospinal fluid (CSF) of patients who developed AD (personal communication from E. van Mechelen, Innogenetics). Overall, our results show that amyloidosis must be completely revisited with this new concept of truncated A β as the cause of amyloidosis in sporadic AD.

Relationship of brain lesion distribution to mild cognitive impairment

Because our prospective study of more than 200 patients led us to collect all data on cognitive status as well as the extent of tau and A β pathology, it was interesting to determine whether there was a relationship between brain lesions and mild cognitive impairment (MCI). The table of all our patients, presented in reference 10, showed in fact that all MCI patients had a tau pathology, but not necessarily

A β pathology. Furthermore, all patients with a mild tau pathology did not have MCI, probably because tau burden can be compensated for in some patients. These results agree with those of recent paper of the Mesulam group,²¹ showing that tau pathology is more closely related to cognitive impairment than is A β . However, longitudinal studies have shown that the additional presence of N-truncated A β deposits in the brain or in the CSF in patients with MCI, and tau pathology is a predictor of an emerging AD (E. van Mechelen, in preparation).

Plaques and tangles: which are the closest to cognitive impairment?

Together, neuropathological and biochemical results show that tau pathology is closer to cognitive impairment than A β deposition. There is an almost general agreement on that point, and this is logical, because tau pathology shows the neuronal networks that are affected. This correlation concerns a rather late stage of the disease. The question of the origin of neurodegeneration, which concerns the very first steps of the disease, is a different issue that was addressed by searching the earliest molecular defects linked to A β and tau aggregation.

RELATIONSHIP BETWEEN TAU PATHOLOGY AND AMYLOID PRECURSOR PROTEIN DYSMETABOLISM

The parallelism and the synergy between tau and A β aggregation led us to search an APP molecular event linking the two degenerating processes. Therefore, we quantified all APP metabolic products in relationship to the different stages of tau pathology.

We did not observe significant changes in full-length APP in Alzheimer patients. However,

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we found that APP carboxy terminal fragments (APP-CTFs), which are released after the action of secretases (α -, β - and γ -secretase activities) upstream of the production of $A\beta$ were significantly modified and decreased at an early stage of AD (Figure 39.1). A significant decrease of the five main APP-CTFs electrophoretic bands was observed, which correlated well with the progression of tau pathology, in most cases with infraclinical AD and with AD (either familial or non-familial). Furthermore, solubility properties and the ratio between the five bands were also modified, in both the Triton-soluble and/or-insoluble fractions. This modification directly observed on APP-CTFs upstream of $A\beta$ products and its relationship with tau pathology could reflect the basic etiological APP dysfunction mechanisms of sporadic AD.²² This abnormal processing of APP-CTFs could be directly responsible for the production of N-truncated $A\beta$ species and amyloidosis.

CONCLUSION

The facts that tau pathology is a neuron-to-neuron spreading phenomenon, and that the

neocortical involvement is always found in the presence of $A\beta$ deposits, demonstrate that: AD is a real tauopathy; there is a synergy between amyloidosis and tauopathy; and the early transformation and decrease of APP-CTFs, in parallel with tau pathology, is in favor of a loss of APP function as the central cause of AD. AD could be explained by the loss of APP-CTFs trophic activities, provoking the extent of tau pathology. APP-CTFs are likely modulating transcription factors.²³ They can be considered as 'survival factors'. Their lack could stimulate the tau degenerating process. Conversely, the microtubule network also has a major importance in regulating the transport and metabolism of APP and associated molecules. Thus, it is also possible that APP dysfunction in sporadic AD is an early consequence of an altered microtubule network, implicating microtubule-associated tau proteins. Therefore, AD is most likely to be the result of a convergence of a tau pathology that occurs frequently in aging, and a defect of APP metabolism, which fuels the spreading of the tauopathy in neocortical areas. Our results suggest that both tau and APP pathologies are targets for diagnosis and therapy.



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