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# Alzheimer Disease, a Multifactorial Disorder Seeking Multi-therapies

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## Abstract

Alzheimer disease (AD) is multifactorial and apparently involves several different etiopathogenic mechanisms. There are at least five subgroups of AD based on cerebrospinal fluid levels of $A\beta_{1–42}$, a marker of Aβ plaques, and tau and ubiquitin, two markers of neurofibrillary tangles. These different AD subgroups may respond differently to a given disease modifying drug and, hence, different therapeutic drugs for different disease subgroups might be required. Stratification of AD patients by disease subgroups in clinical trials is critical to the successful development of potent disease modifying drugs. Cerebrospinal fluid levels of disease markers are promising, both in identifying various subgroups of AD and in monitoring the response to therapeutic drugs.

### Keywords

Alzheimer disease subgroups; cerebrospinal fluid; CSF biomarkers; $A\beta_{1–42}$; tau; ubiquitin; Alzheimer disease therapeutics; neurofibrillary degeneration; tau; β-amyloid

## 1. Introduction

Alzheimer disease (AD), the single major cause of dementia in middle and old age individuals, is histopathologically characterized by brain β-amyloidosis (Aβ) and neurofibrillary degeneration. The former is seen as plaques of extracellular deposits of Aβ in the brain parenchyma and in the cerebral blood vessels, the congophilic angiopathy. The neurofibrillary degeneration is a slow and progressive retrograde neuronal degeneration that is observed as neurofibrillary tangles of paired helical filaments (PHF)/straight filaments (SF) in the cell soma, and in dystrophic neurites surrounding the plaque core β-amyloid, and in the neuropil as neuropil threads [1]. Aβ plaques identical to those in AD but lacking the dystrophic neurites with neurofibrillary pathology are also seen in the neocortex of the cognitively normal old age individuals [2]. On the other hand, neurofibrillary pathology of the AD type which is made up of PHF/SF of abnormally hyperphosphorylated tau [3,4] is a hallmark of several related neurodegenerative diseases called tauopathies (for review see [5]. These tauopathies include frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) caused by tau mutations, corticobasal degeneration, Pick disease, dementia pugilistica, and progressive nuclear palsy. The occurrence of neurofibrillary degeneration in the neocortex in the absence of Aβ deposits in tauopathies is associated with dementia. In progressive supranuclear palsy



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where the hyperphosphorylated tau lesions occur in the brain stem, motor dysfunction instead of dementia is observed. Unlike aging and tauopathies, AD is characterized by the presence of both numerous β-amyloid plaques and neurofibrillary tangles of abnormally hyperphosphorylated tau filaments in the neocortex, especially the hippocampus. The abnormally hyperphosphorylated tau in neurofibrillary tangles becomes ubiquitinated [6,7]. However, this ubiquitination occurs late, i.e. when the pathological tau is in β-pleated sheets, and is mostly unsuccessful; neurons with ubiquitinated neurofibrillary tangles survive for up to several years [8] and then on cell death are seen in the extracellular space in the brain as ghost tangles, also called tombstones.

## 2. Multifactorial nature and a lack of apparent temporal relationship between plaques and tangles

AD is multifactorial and heterogeneous. Less than 1% of AD cases are caused by certain mutations in three different transmembrane proteins, amyloid precursor protein (APP), presenilin 1 and presenilin 2 (for review see [9]). Furthermore, prion protein, the self-replication of the pronase resistant form of which causes prion disease, is also a transmembrane protein, and a missense mutation in this protein in Gerstman Straussler Syndrome (GSS) in a family in Indiana, referred to as Indiana kindred, has been found to be associated with numerous neurons with neurofibrillary tangles of abnormally hyperphosphorylated tau along with prion plaques [10]. Over 99% of AD cases represent the so called sporadic form of the disease which is not associated with any known mutation. The sporadic form of AD itself probably involves several different etiopathogenic mechanisms. Neuroinflammation, head trauma, and diabetes have been implicated as risk factors for AD. In the sporadic AD, the presence of one or two alleles of $APOE_4$ as opposed to $APOE_2$ or $APOE_3$ increases the disease risk by several fold [11]

Although numerous plaques and neurofibrillary tangles are seen in AD brain, for reasons currently not understood these two lesions occur in disproportionate numbers in different cases of the disease, especially in the plaque-dominant and tangle-dominant AD subgroups [12,13]. This lack of direct relationship between the numbers of plaques and tangles in AD and the presence of numerous Aβ plaques without accompanying neurofibrillary degeneration in normal aged humans are inconsistent with the Amyloid Cascade Hypothesis [14,15], according to which Aβ, the metabolite of the β-amyloid precursor protein (βAPP), is the primary neurotoxic molecule which causes neurofibrillary degeneration and leads to dementia. In support of this hypothesis, infusion of $Aβ_{1–42}$ in P301L tau transgenic mice [16] as well as crossing of P301L tau transgenic mice with $APP_{SWE}$ transgenic mice [17] have been shown to exacerbate the tau neurofibrillary pathology. In these studies, the infusion of $Aβ_{1–42}$ or overexpression of $APP_{SWE}$ could have exacerbated the tau pathology by activating the stress activated protein kinases which are known to phosphorylate tau at several proline-directed sites. However, no mutations in tau have been found to date in AD and the FTDP-17/tau mutation cases do not show any Aβ deposits. Furthermore, numerous Aβ plaques in the neocortex of normal aged humans in the absence of neurofibrillary pathology [2] and a very high Aβ load in hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D) but without accompanying neurofibrillary pathology [18] occur. Thus, the Amyloid Cascade Hypothesis does not seem to hold in human brain.

Two AD Phase III clinical trials using an Aβ aggregation inhibitor, Alzhemed, and another using a γ-secretase modulating non-steroidal anti-inflammatory drug (NSAID) Flurizine (Myriad, Utah), have badly failed. A clinical trial on direct removal/clearance of β-amyloid from the brains of AD patients by Aβ vaccine (AN1792 by Elan Pharm., Ireland) had to be interrupted due to the vaccine-induced menigoencephalitis in several participants. Nevertheless, the Aβ vaccine though successful in clearing β-amyloid from the brain

parenchyma of the treated AD cases studied postmortem, this treatment had failed to reduce the number of neurofibrillary tangles and significantly alter the cognitive decline [19]. Lastly, a Phase II clinical trial of AD patients treated by passive immunization with monoclonal antibody to Aβ (Elan/Wyeth Pharm) failed to show any significant clinical improvement. Thus, it appears that either Aβ deposition is not the primary cause of dementia or inhibition or clearance of Aβ without inhibition of neurofibrillary degeneration might not be sufficient to inhibit the progressive cognitive decline in AD patients. Another possibility is that Aβ initiates neurofibrillary pathology which then becomes self-propagating. In such a case, removal/clearance of Aβ from AD brain could be too late to be of any clinical benefit. However, the presence of numerous Aβ plaques without accompanying neurofibrillary pathology in the neocortex of cognitively normal aged humans and in HCHWA-D cases does not support this scenario.

Failures with Aβ-based therapies have shifted interest to developing therapeutic drugs that can inhibit Alzheimer neurofibrillary degeneration. The development of drugs that can inhibit neurofibrillary degeneration has its own challenges as well as opportunities. Neurofibrillary degeneration of AD type can result from several different etiopathogenic mechanisms and, thus, offers many therapeutic targets.

Tau protein has 80 serines/threonines and 5 tyrosines as potential sites that can be phosphorylated by protein kinases [20]. Normal brain tau has 2–3 moles phosphate/mole of the protein and this stoichiometry is apparently optimal for its biological activity which is regulated by its degree of phosphorylation. By hyperphosphorylation a neuron can reduce the microtubule network and the axonal transport and other cellular activities that depend on microtubules. However, this is reversible and plays a physiologically significant role. It is important to distinguish this type of hyperphosphorylation of tau from that which occurs in AD and related tauopathies. Two main characteristics of the AD abnormally hyperphosphorylated tau (AD P-tau) that were discovered in our lab are (i) that instead of interacting with tubulin, the AD P-tau binds to normal tau, MAP1 and MAP2, and this sequestration of normal MAPs results in depolymerization of microtubules [21–24], and (ii) that the AD P-tau can self-assemble into bundles of PHF/SF [25,26].

Phosphorylation sites that lead to the AD type abnormal hyperphosphorylation of tau are phosphorylated by different combinations of non-proline-directed protein kinases like PKA and CaMKII with the proline directed kinases like glycogen synthase kinase 3β (GSK-3β), and cyclin dependent protein kinase 5 (cdk5), and are dephosphorylated largely by protein phosphatase-2A [26]. Furthermore, phosphorylation of tau is also regulated by its glycosylation which is altered in AD brain [27,28]. These studies suggest that different etiopathogenic mechanisms could be involved in the abnormal hyperphosphorylation of tau.

Tau self-assembles through the β structure containing microtubule binding repeat R3 in 3R taus and through repeats R2 and R3 in 4R taus [29,30]. Both the amino terminal and the carboxy terminal flanking regions to the microtubule binding repeats in normal tau appear to inhibit its self-assembly. Whereas on AD-type abnormal hyperphosphorylation, i.e. the phosphorylation of the amino terminal and carboxy terminal flanking regions, this inhibition is eliminated, resulting in the self-assembly of tau into tangles of PHF/SF [31,32]. In addition to abnormal hyperphosphorylation, truncation of tau at Glu391 and Asp421 have been implicated in the pathogenesis of AD [33–35]. Truncation probably promotes self-aggregation of tau both by decreasing the inhibitory effect of the carboxy terminal domain in the case of $tau_{391}$ and $tau_{421}$, as well as by making the truncated proteins better substrates for phosphorylation. Transgenic rats expressing human tau truncated both N- and C-terminally, $tau_{151–391}$, show marked neurofibrillary degeneration of abnormally hyperphosphorylated tau [36].

## 3. Different subgroups of AD

Based on the CSF levels of $A\beta_{1–42}$, tau and ubiquitin studies from our lab led to the identification of five different subgroups of sporadic AD [37]. These five subgroups, called AELO, ATEO, LEBALO, HARO, and ATURO, each present a different clinical profile. Thus, it is likely that each of these sporadic AD subgroups may respond differently to any one given disease modifying drug. The subgroup AELO, which represents AD cases with low $A\beta_{1–42}$ level, high incidence of $APOE_4$, and late onset, represented almost half of the 353 AD cases examined in our study [37]. Two AD Phase II clinical trials, one with Rosiglitazone [38], also an anti-diabetic drug (Smith Kline Glaxo, USA), and another with Aβ passive immunization (Elan/Wyeth Pharm., USA), both showed a better treatment response to $APOE_4$ non-carriers as compared with $APOE_4$ carriers. It thus indicates that AD subgroup AELO is likely to respond poorly to Rosiglitazone and to Aβ vaccine.

The subgroup HARO, which represents AD cases with high instead of low CSF $A\beta_{1–42}$ seen in the other four subgroups, is characterized by recent onset and represented <10% of the cases in our study, is likely to respond very differently from the rest of the AD cases to Aβ-based therapies. Similarly, the subgroup LEBALO, that represented AD cases with high incidence of Lewy bodies, low $A\beta_{1–42}$ and late onset and constituted ~15% of the cases in our study, did not have any significantly elevated CSF tau and thus is very likely to respond very differently from the rest of the AD cases to a tau-based therapeutic drug. Thus, our current preliminary knowledge on different subgroups of AD and different response of $APOE_4$ carriers vs. non-carriers, mentioned above, strongly suggests that the success of clinical trials of AD for disease modifying drugs can be markedly improved by stratifying test patients by subgroups. Future studies on CSF molecular markers, especially levels of different phosphotaus, may help identify additional specific subgroups of AD. Stratification of AD cases by these additional subgroups is likely to markedly increase the success of developing potent disease modifying drugs for this disease.

## 4. Conclusions

AD is multifactorial and heterogeneous and involves several etiopathogenic mechanisms. There are at least five subgroups of AD that can be identified by determining CSF lavels of $A\beta_{1–42}$, tau, and ubiquitin. Stratification of AD patients by these subgroups and monitoring the efficacy of the drug treatment by CSF levels of the disease markers are promising approaches for the development of rational disease modifying drugs.

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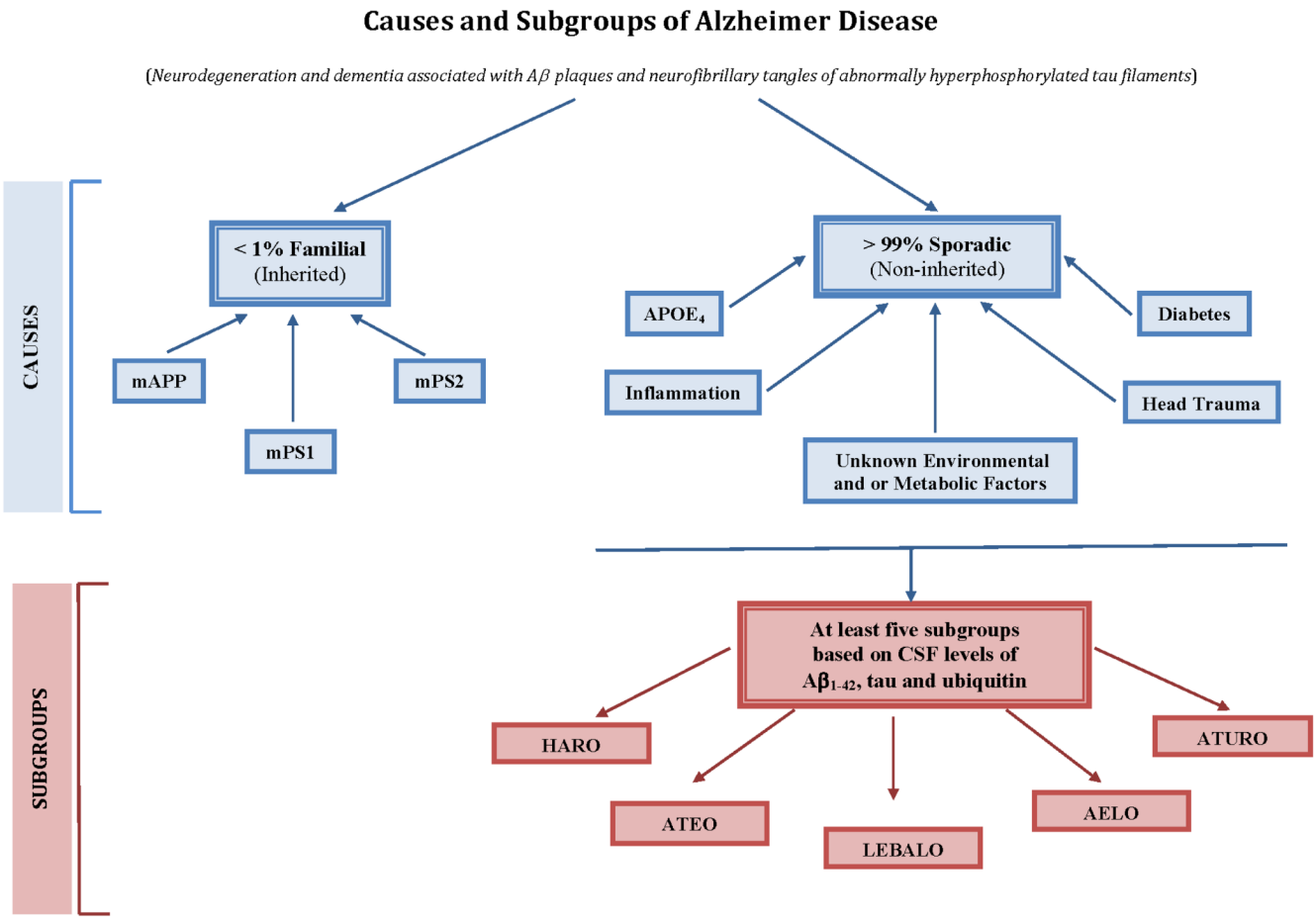


**Figure 1. Causes and subgroups of AD**

Less than 1% of AD cases are familial and are caused by certain mutations in βAPP (mAPP), presenilin 1 (mPS1), or presenilin 2 (mPS2) genes which are transmitted in an autosomal dominant fashion and the resulting gene products, the mutated proteins, are dysfunctional. Over 99% of AD cases are sporadic and have not, to date, been associated with any mutated protein, but several risk factors including the presence of one or two copies of $APOE_4$ allele, inflammation, head trauma, diabetes/low brain glucose metabolism, and as yet unknown environmental and or metabolic factors have been implicated.

Based on CSF levels of $A\beta_{1–42}$, a marker of Aβ plaques, and tau and ubiquitin, the two markers of neurofibrillary tangles, five subgroups of AD have been identified. These subgroups, AELO, ATEO, LEBALO, HARO, and ATURO, represent distinct clinical profiles. These different subgroups of AD probably involve different etiopathogenic mechanisms and may respond differently to any one given disease modifying drug.

**AELO**: AD cases with low $A\beta_{1–42}$, high incidence of $APOE_4$, and late onset.
**ATEO**: AD cases with low $A\beta_{1–42}$, high tau, and early onset.
**LEBALO**: AD cases with high incidence of Lewy bodies, low $A\beta_{1–42}$, and late onset.
**HARO**: AD with high $A\beta_{1–42}$, and recent onset.
**ATURO**: AD with low $A\beta_{1–42}$, high tau, high ubiquitin, and recent onset.