

# Molecular mechanisms of neuropathological changes in Alzheimer's disease: a review

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## Abstract

More than 100 years after description of Alzheimer's disease (AD), two major pathological processes observed already by Alois Alzheimer, remain as the main explanation of the pathogenesis of Alzheimer's disease. Important molecular interactions leading to AD neuropathology were described in amyloid cascade and in tau protein function. No clinical trials with novel therapies based on amyloid cascade and tau protein hypotheses have been successful. The main aim of recent research is focused on the question what is primary mechanism leading to the molecular development of AD pathology. Promising explanation of triggering mechanism can be seen in vascular pathology that have direct influence on the development of pathological processes typical for Alzheimer disease. Novel insight into a number of cellular signaling mechanisms, as well as mitochondrial function in Alzheimer disease could also bring explanations of initial processes leading to the development of this pathology.

**Key words:** amyloid precursor protein, tau protein, apolipoprotein E, secretase, mitochondria, vascular.

## Introduction

Alzheimer's disease (AD) was described as "presenile dementia" first in 1906 by German psychiatrist Alois Alzheimer, colleague of Emil Kraepelin. In 1901, Alzheimer observed a patient named Auguste D. with a progressive loss of cognitive functions (comprehension and memory, unpredictable behaviour etc.). Auguste D. died in April 1906. Alzheimer analysed her brain postmortem using histological methods and wrote in the description "Numerous small miliary foci are found in the superior layers. They are determined by storage of peculiar

material in the cortex" [39]. Alzheimer continued: "all in all we have to face a peculiar disease process. Such peculiar disease processes have been verified recently in considerable numbers". "Miliar foci, which are caused by deposition of a peculiar substance in the cortex" are recognised today as senile plaques and "very peculiar changes in the neurofibrils" are recognised today as helical tangles. Emil Kraepelin introduced the eponym "Alzheimer's disease" for "presenile dementia" [39]. More than 100 years after describing of Alzheimer's disease, two major pathological processes (amyloid beta and tau protein deposition) observed already by Alois

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Alzheimer remain the main explanation of pathogenesis of Alzheimer's disease even though some other very important molecular, genetic and epidemiological hypotheses were expressed [53]. The main problem of the explanation of pathophysiology of Alzheimer's disease still could be seen in the inability to identify key mechanisms that release pathologies observed in AD.

## Amyloid cascade hypothesis

The human amyloid precursor protein (APP) was first identified in 1987 by several laboratories [20,64,57]. The APP gene was then mapped to chromosome 21 [58]. It has been determined that the APP gene contains 19 exons and spans more than 170 kb [74]. APP has several isoforms generated by alternative splicing of exons 1-13, 13a, and 14-18. The predominant transcripts are APP695 (exons 1-6, 9-18, not 13a), APP751 (exons 1-7, 9-18, not 13a), and APP770 (exons 1-18, not 13a).

Amyloid precursor protein is type I transmembrane protein. Amyloid precursor protein is synthesized in the endoplasmic reticulum and then transported through the Golgi apparatus to the trans-Golgi network (TGN) where it is stored to its higher concentration at steady state [66].

Over 32 different APP missense mutations have been identified in 85 families [6]. Mutations within APP account for 10% to 15% of early-onset familial AD. Most cases containing APP mutations have an age of onset in the mid-40s and 50s [23]. It was observed that patients with Down syndrome (trisomy 21) develop amyloid deposits. Amyloid deposits cause the neuropathological features of AD patients when they are in their 40s. Three morphological subtypes of amyloid deposit are observed in the brain of AD patients: a) diffuse deposits, in which A $\beta$  peptide is not aggregated into the amyloid, b) primitive deposits, in which the A $\beta$  peptide is aggregated into the amyloid and associated with dystrophic neuritis and helical filaments, and c) classic deposit, in which A $\beta$  is highly aggregated to form a central amyloid "core" surrounded by a "ring" of dystrophic neuritis [3]. Armstrong [3] found a larger average cluster size of the diffuse deposit in patients with a familial form of Alzheimer's disease in comparison with patients with sporadic Alzheimer's disease. Presented evidence suggests that postmenopausal oestrogen replacement therapy may prevent or delay the onset of AD [67]. It has been determined that the beneficial effect of oestrogen is mediated by accelerated

trafficking of beta APP through the trans-Golgi network (TGN), which precludes maximal beta-amyloid production [21].

Amyloid precursor protein can be processed at different cleavage sites by different proteases to few peptides with biological functions. Amyloid precursor protein undergoes posttranslational proteolytic processing by  $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretases.  $\alpha$ -secretase generates soluble amyloid protein, while  $\beta$ - and  $\gamma$ -secretases generate APP components with amyloidogenic features.

## $\alpha$ -secretase

Amyloid precursor protein processing by  $\alpha$ -secretase precludes the production of small peptides called  $\beta$ -amyloid. Amyloid precursor protein is delivered to the plasma membrane by the cytoskeletal system where it is subjected to proteolytic processing by  $\alpha$ -secretase. A soluble molecule named sAPP $\alpha$  is released after this cleavage. sAPP $\alpha$  has an important role in neuronal plasticity/survival and it is protective against excitotoxicity. sAPP $\alpha$  also regulates neural stem cell proliferation and is important for early CNS development [48].

$\alpha$ -secretase is a zinc metalloproteinase that is also type-I transmembrane protein. The family of proteins with  $\alpha$ -secretase activity includes ADAM9, ADAM10 and ADAM17. Constitutive  $\alpha$ -secretase is ADAM10 [33]. Disruption of ADAM10 activity has been shown to decrease the level of soluble non-amyloidogenic APP, suggesting that maintaining ADAM10 activity may play a protective role in Alzheimer's disease for processing of APP via the  $\alpha$ -secretase pathway. Biologically important substrates of ADAM10 include the epidermal growth factor (EGF), betacellulin, Notch, and amyloid precursor protein (APP) [40]. Two potentially pathogenic mutations with incomplete penetrance for late-onset familial AD in the ADAM10 gene were described and it has been found that ADAM10 has  $\alpha$ -secretase activity that mediates the effect of cholesterol (influence of apolipoprotein E) on APP metabolism [30,32]. Treatment of various peripheral and neural human cell lines with either a cholesterol-extracting agent or an HMG-CoA reductase (HMGCR) inhibitor resulted in a drastic increase of secreted alpha-secretase-cleaved soluble APP peptides. It has been demonstrated that cholesterol reduction promotes the non-amyloidogenic alpha-secretase pathway and the formation of neuroprotective alpha-secretase cleaved soluble APP by several mechanisms [32].

## $\beta$ -secretase

Absence of the  $\alpha$ -secretase cleavage leads to APP molecules internalization into endocytic compartments where they are subjected to cleavage by  $\beta$ - and  $\gamma$ -secretases to generate A $\beta$ . Amyloid precursor protein  $\beta$ -secretase 1 (BACE1) was identified and described [70,73].  $\beta$ -secretase 1 is  $\beta$ -secretase involved in APP metabolism.  $\beta$ -secretase 1 is a membrane-bound aspartyl protease with a characteristic type I transmembrane domain near C-terminus. The BACE gene is located on chromosome 11 and consists of nine exons coding for a protein of 501 amino acids [64].  $\beta$ -secretase 1 pre-mRNA can undergo alternative splicing in exons 3 and 4, which results in the production of four alternative variants with 501, 476, 457, and 432 amino acids. The shorter splice variants have little cleavage activity on APP substrate compared to the 501 amino acid protein [41]. Precursor of BACE1, named pro-BACE1 is modified by glycosylation, phosphorylation and then cleaved by a furin-like endoprotease to produce mature BACE1. After synthesis in the endoplasmic reticulum, BACE1 is transported through the secretory route to the plasma membrane from where it is re-internalized into endosomal compartments [55].  $\beta$ -secretase 1 requires acidic environment for optimal activity. This optimal environment is provided by endosomes.

$\beta$ -secretase expression increases with age [18], and is particularly elevated in the brain cortex of AD patients [24]. Several mechanisms have been proposed to explain this increase [26]. A defect in BACE trafficking due to caspase degradation of the GGA that controls BACE intracellular sorting, or a loss of control of BACE mRNA translation have been proposed as mechanisms to explain age-dependent increase of BACE expression. Oxidative stress and other conditions such as hypoxia, ischaemia, and energy deprivation have also been found to elevate BACE expression in cellular models [22].  $\beta$ -secretase 1 is still recognised as the drug target for the treatment of Alzheimer's disease even though many important proteins are additional BACE1 substrates, e.g. low-density lipoprotein receptor/related protein, P-selectin glycoprotein ligand/1, neuregulin (Nrg1-type III  $\beta$ 1, and Nrg3) and the  $\beta$ 2 subunit of voltage-gated sodium channel (Na $_v$ 1,  $\beta$ 2), some of which play an important role in the development and normal function of the brain [16].  $\beta$ -secretase 2 (BACE2) is another  $\beta$ -secretase that cleaves APP near the  $\alpha$ -secretase site more efficiently than BACE1 and this suggest that BACE1 is primary  $\beta$ -secretase.

After APP cleavage by BACE1, ectodomain of APP is released as a soluble peptide named sAPP $\beta$ . Region 1-16 of carboxyl-terminus that lacks sAPP $\beta$  is the difference between sAPP $\alpha$  and sAPP $\beta$ . The role of both peptides is dramatically different. sAPP $\beta$  has a function as ligand of death receptor 6, mediates axonal pruning and neuronal cell death [46].

After  $\alpha$ - and  $\beta$ -cleavage, the carboxyl terminal fragments (CTFs) of APP described as  $\alpha$ CFT and  $\beta$ CFT remain membrane-associated and will be further cleaved by  $\gamma$ -secretase. Overproduction of  $\beta$ CFT has a cytotoxic effect and causes neuronal degeneration. It could be also done by cytotoxic peptides C31 and Jcasp that arise from cleavage of  $\beta$ CFT by  $\gamma$ -secretase or by caspase, including APP intracellular domain [49].

## $\gamma$ -secretase

$\alpha$ CFT is processed by  $\gamma$ -secretase to p83 peptide that is rapidly degraded and its function was not described.  $\beta$ CFT is cleaved by  $\gamma$ -secretase to A $\beta$ 40 and A $\beta$ 42. Recently, other sites cleavage by  $\gamma$ -secretase have been described –  $\zeta$ -site (A $\beta$ 46) and  $\epsilon$ -site (A $\beta$ 49).  $\alpha$ CFT is then processed sequentially in  $\epsilon$ -site,  $\zeta$ -site and finally in  $\gamma$ -site [75].

$\gamma$ -secretase is a big complex composed from a few components, mainly from four proteins: presenilin (PS, PS1 or PS2), nicastrin, anterior pharynx-defective-1 (APH-1) and presenilin enhancer-2 (PEN-2).  $\gamma$ -secretase complex is located in endoplasmic reticulum, Golgi complex and trans-Golgi network, endocytic and intermediate compartments [61].

In human, two homologues of presenilin were described – presenilin 1 and presenilin 2. Mutations in PS1 (and also PS2) were described in the familial form of AD. More than 1000 point mutations in the presenilins are responsible for most of the familial forms of AD [47].

## Amyloid precursor protein trafficking

Amyloid precursor protein is biosynthesized in endoplasmic reticulum and anterogradely transported to the Golgi apparatus and then to the trans-Golgi network in distinct transport vesicles by conventional kinesin. In the trans-Golgi network APP undergoes various post-translational modifications (phosphorylations, tyrosine sulfations and *N*- and *O*-glycosylations). Recently, calsyntenin-1 has been shown to be co-transported with APP along axons [69]. Calsyntenin-1 brain cellular level is reduced in persons with Alzheimer's disease and

the extent of calyntenin-1 reduction correlates with increased A $\beta$  levels. GTPase activity of Rab3, a small G-protein of the Rab family that is involved in the late steps of exocytosis, is required for APP transport vesicles [65].

After insertion of APP at the plasma membrane it undergoes rapid clathrin-mediated endocytosis. C-terminal of APP with four amino acid sequence, YENPY, is the major signal for clathrin-mediated APP endocytosis [50]. Many intracellular adaptors bind to this C-terminal part of APP, for example Fe65, Mint proteins, Dab1 or JIP [11]. Lipoprotein receptor-related protein (LRP1) and apolipoprotein E receptor 2 are able to interact directly with APP. Depending on the linker protein involved, apolipoprotein E receptor 2 and APP are connected intracellularly via Dab1 (disabled family member), Mint1 or Fe65 adaptors or extracellularly by F-spondin [11]. There are alternative ways of APP after internalization. Amyloid precursor protein can be transported rapidly and directly from the cell surface to lysosomes [36]. Amyloid precursor protein can be also degraded in proteasome [9]. Amyloid precursor protein can be transported from endosomes to Golgi apparatus and/or TGN from where it could be distributed back to plasma membrane.

## A $\beta$ function

It has been shown that the extracellular domain of APP is especially important for promoting synapse formation. Trans-synaptic interactions between pre- and postsynaptic APP contribute to the adhesion of synapses [72]. It was found that APP knock-out and also BACE1 knock-out mice show impaired memory [31]. It suggests a necessary role of A $\beta$  in learning and memory. Recently, it was found that a low level of A $\beta$  increases hippocampal long-term potentiation and enhances memory, indicating a novel positive, modulatory role on neurotransmission and memory [54]. Picomolar A $\beta$  is present in both the cerebrospinal fluid and plasma of healthy individuals throughout life. It has been shown that picomolar concentrations of both A $\beta$  42 monomers and oligomers cause a marked increase in long-term potentiation, whereas high nanomolar concentrations lead to the well-established reduction of potentiation in the hippocampus [54].

There are two main toxic species of A $\beta$  – A $\beta$ 40 and A $\beta$ 42. The increase in the ratio of A $\beta$ 42/A $\beta$ 40 is typical of AD patients [62]. Majority of A $\beta$  peptides is secreted from the neurons as A $\beta$ 40. A smaller fraction of A $\beta$ 42

is cleaved to produce A $\beta$ 42 that is the main amyloid peptide that is responsible for the production of amyloid fibrils in AD patients. A $\beta$ 42 self-associates to dimers, soluble oligomers and to insoluble aggregates of fibrils. Extracellular A $\beta$  can be internalized by cells for intracellular degradation, e.g. by insulin-degrading enzyme and neprilysin. Presented data imply a mechanism for the formation of A $\beta$  amyloid plaques in which initially soluble and extracellular A $\beta$  peptide becomes internalized and sorted into multivesicular bodies [17]. Upon spontaneous nucleation or in the presence of suitable fibril seeds, fibrils grow out, disturb the ordered multivesicular bodies function and penetrate the vesicular membrane. Ultimately cells die and all intracellular structures, including all intracellular amyloid species, become released into the extracellular space [17]. The cytotoxic effect of A $\beta$  could be achieved also by the above described C31 and Jcasp peptides released during  $\beta$ CFT cleavage.

There have been more than 30 investigations assessing plasma amyloid beta A $\beta$ 40 and A $\beta$ 42 as a diagnostic or as a biological risk factor. A $\beta$ 42 and A $\beta$ 42/A $\beta$ 40 ratio levels were elevated in unaffected familial AD mutation carriers compared with unaffected individuals with familial AD without mutations [56]. However, A $\beta$ 42 levels were lower in mutation carriers with incipient AD characterized as having a clinical dementia rating (CDR) = 0.5 [25], supporting the hypothesis that A $\beta$ 42 decreases prior to overt disease.

Recently, the transfer of A $\beta$  between neurons has been described. This transfer is dependent on the synaptic connection between neurons [44]. Previously, the degeneration of entorhinal cortex in the initial stage of AD and subsequent degeneration of connected areas was described. The exogenous intracerebral injection of A $\beta$  aggregates taken from brain extracts of AD patients induced cerebral amyloidogenesis that progresses from the injection site in APP transgenic mice. Newly described transfer of A $\beta$  between neurons could explain spreading the neurodegenerative pathology to anatomically connected brain areas [44].

## Tau protein (microtubule-associated protein tau, MAPT)

The human gene for tau protein (MAPT gene) is located on chromosome 17 [45]. It contains 15 exons. Exons 2, 3 and 10 are alternatively spliced resulting in six isoforms. There are 79 potential serine and threonine phosphate acceptor residues in the longest iso-

form of tau. Tau has more than 30 phosphorylated sites. Normal tau protein stabilizes microtubules in the cytoskeleton of neurons, promote neurite outgrowth, membrane interactions, facilitate enzyme anchoring and facilitate axonal transport of organelles to nerve terminals [27]. The phosphorylation of tau protein regulates microtubule binding and assembly [71]. It has been demonstrated that in solution normal tau associated with the hyperphosphorylated tau protein to form large tangles of filaments [1]. In AD, tau protein is hyperphosphorylated then it accumulates in neurons and forms paired helical filaments. Tau protein loses its capability to bind with microtubules and it leads to neurodegeneration. Astrocytes are essential for the A $\beta$ -induced tau phosphorylation observed in primary neurons [19].

Causal factors affecting phosphorylation of tau are not fully understood but according to observation, multiple factors in this process are expected.

Abnormal binding of hyperphosphorylated tau protein on microtubules typical of AD patients causes instability of microtubules and lead to abnormal axonal transport that is dependent on microtubules. A $\beta$  and mitochondria are transported along microtubules by molecular motors. Inhibition of axonal transport leads to accumulation of APP in cell body. It was found that impaired axonal transport of organelles including mitochondria causes oxidative stress [38].

In the tau gene no mutation related to AD was found. There was reported the association between the H1c subhaplotype of the MAPT gene and the risk of Alzheimer's disease in 360 autopsy-confirmed cases with ages at death over 65 years of age and 252 controls [42].

It was demonstrated that the MAPT gene rs242557 polymorphism that is part of the H1c subhaplotype, results in increased MAPT gene expression [34]. The authors also provided evidence that the H1/H2 MAPT haplotype interacts with functional SNPs in the GSK3B gene to affect the risk of Alzheimer's disease.

The relationship between tau protein and mitochondria was recently described. Tau protein was found on mitochondrial membranes. It is increasingly accepted that the trafficking to, and density of, mitochondria at subcellular locations with the energy and Ca<sup>2+</sup>-buffering requirements, including synapses, is important for correct neuronal function [37]. The distribution of mitochondria in axons and dendrites correlates closely with the predicted energy usage of these compartments. Mitochondria undergo rapid trafficking in axons and dendrites. Synaptic activity modulates mitochondrial

motility and morphology and controls mitochondrial distribution in dendrites and their recruitment to the base of dendritic spines [35]. In the experiment conducted on neurons transfected with tau protein, mitochondria disappeared from the neurites and became concentrated in the cell body [63]. Preferential inhibition of plus-end-directed transport (outside the cell centre) of mitochondria and other organelles by kinesin molecular motors was observed as a result of tau protein level elevation. Minus-end-directed transport (inside the cell centre) by a dynein-like motor then becomes dominant [63].

## Apolipoprotein E

Apolipoprotein E is a cholesterol transport protein. It can be found mainly as a component of lipoprotein complexes along with other apolipoproteins and proteins in plasma and CSF. Three alleles ( $\epsilon$ 2 – Cys112/Cys158,  $\epsilon$ 3 – Cys112/Arg158 and  $\epsilon$ 4 – Arg112/Arg158) were described in humans, according to combinations in two polymorphic sites. Amino acid differences at these positions are crucial as they alter the charge and structural properties of the protein.

The ApoE gene has been associated with both familial late-onset and sporadic late-onset AD in numerous studies. To date only ApoE4 has been firmly identified as a genetic risk factor, although segregation analyses conducted in families of patients with LOAD support the presence of additional genetic variants [13]. With a population attributable risk that is estimated at 20-50%, the ApoE4 allele increases the risk of cognitive impairment, LOAD, and age-of onset of cognitive impairment in a dose-dependent fashion: 1  $\epsilon$ 4 allele is associated with a 2- to 3-fold increased risk, having 2 copies is associated with a 5- to 10-fold increase. Similar effect sizes have been observed for progression of cognitive impairment to dementia. Individuals carrying ApoE4 allele have higher total and LDL cholesterol [60].

High cholesterol levels have been linked to overproduction of A $\beta$ . One of the physiological functions of A $\beta$  has been suggested to control cholesterol transport. Individuals of 50 years and older who were prescribed statins had a substantially lowered risk of developing dementia, independent of the presence or absence of untreated hyperlipidaemia, or exposure to non-statin LLAs [28]. Cholesterol greatly reduced the levels of sAPP $\alpha$ . ADAM10 is unable to cleave APP in a cholesterol-rich environment [32]. Changes in cellular cholesterol levels in Alzheimer's disease could contribute

to neuronal degeneration by decreasing the production of sAPP $\alpha$  [7].

### Possible mechanisms triggering Alzheimer's disease pathology

Vascular and mitochondrial hypotheses of pathogenesis of AD were also stated. Several vascular risk factors e.g. diabetes mellitus, hypertension, atherosclerosis, hypercholesterolemia, metabolic syndrome and obesity, have been found to be associated with Alzheimer's disease [51,52]. The apolipoprotein E genotype with the link to dynamics of cholesterol transport is also implicated as a vascular risk factor in influencing AD [29]. AD patients often exhibit various cerebrovascular pathologies including cerebral microbleeding [14,15,43] and cerebral microinfarcts. Microinfarcts are common in patients with vascular dementia (weighted average 62%), Alzheimer's disease (43%), and demented patients with both Alzheimer-type and cerebrovascular pathology (33%) compared with nondemented older individuals (24%) [8]. Cerebral hypoperfusion may initiate and/or accelerate the neurodegeneration cascade causing amyloid deposition, synaptic and neural dysfunction and lead to cognitive impairment [29,51,52]. A $\beta$  deposition into the capillary wall is strongly associated with the ApoE4 allele as a risk factor [4]. Oxidative stress that can be influenced by hypoxia and also by mitochondrial dysfunction is associated with AD pathogenesis.

Mitochondrial dysfunction relationship with AD could be explained by abnormalities in mitochondrial metabolism, biogenesis, axonal transport, fusion and fission processes and by autophagy [10,12]. Functional mitochondria are supplied to the synaptic terminals by anterograde transport by microtubule associated protein kinesin and dysfunctional mitochondria are transported back to cell soma by dynein [59]. Tau protein has been implicated in abnormal mitochondrial trafficking when hyperphosphorylation of Tau protein negatively affects the transport of mitochondria to synapses and back. The lack of ATP energy in synaptic terminals affects synaptic function and it leads to synaptic damage. The accumulation of transmembrane arrested APP block protein translocation, disrupts mitochondrial function, and impair brain energy metabolism [5]. It has been shown that the interaction between A $\beta$  and NH<sub>2</sub>-tau fragment inhibit the mitochondrial adenine nucleotide translocator-1 (ANT-1) [2]. ANT-1 has a function in export of mitochondrial ade-

nosine triphosphate into the cytosol and has a role in the regulation of the intrinsic apoptosis pathway.

### Conclusions

Alzheimer's disease (AD) is the most common cause of dementia, affecting more than 10% of people over the age of 65. Although considerable progress in the understanding of the molecular mechanisms of the pathogenesis of AD has been made, many aspects, especially key mechanisms that release pathologies, remain controversial. Promising research is focused on the research of hypoxia and oxidative stress caused by different mechanisms, e.g. by vascular and mitochondrial pathologies. A $\beta$  deposition in different cell compartments and in extracellular areas and its pathophysiological role remains to be explained in relationship to other molecular mechanisms. It could be concluded that up to date we know many mechanisms that could affect set up and progress of AD pathogenesis. It seems like AD is not only one or two types of diseases but it could be a group of diseases with similar APP and Tau pathologies that are triggered by different mechanisms. Genetic disposition to AD would play an important role in the mechanisms of Alzheimer's disease initiations. Thus, we could expect a group of diseases specified as Alzheimer's disease when the interplay of environmental and genetic factors would be responsible for the type of initiation of AD pathogenesis.

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