

Review

Alzheimer's Disease: A General Introduction and Pathomechanism

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Abstract. Alzheimer's disease (AD) is the most common form of dementia, which affects more than 35 million people worldwide with increasing tendency. Satisfying therapies and prevention are not available. Since the first description of the fatal progressive neurodegenerative disease in 1907, however, major findings on the molecular mechanisms have been reported. Current clinical trials target diverse aspects and principles of AD, such as the generation and aggregation of amyloid- β (A β). Extracellular amyloid plaques, predominantly consisting of A β , and intracellular neurofibrillar tangles, formed by hyperphosphorylated tau, are the major pathological hallmarks in the brain of AD patients. AD is consequently one of about 40 identified amyloidoses – protein misfolding diseases, which share as their main pathogenic mechanism the aberrant deposition of endogenous proteins as amyloid fibrils. This article aims principally to introduce AD and its identified key players, to summarize classic and recent publications on the complex molecular mechanisms underlying the disease, and to discuss challenges that need to be faced for the development of improved therapeutic strategies.

Keywords: Alzheimer's disease, amyloid- β , amyloid- β protein precursor, fibrils, protein misfolding, oligomers, neurotoxicity, tau

INTRODUCTION TO ALZHEIMER HISTORY

Alzheimer's disease (AD) is the most common form of dementia, accounting for 60–80% of all cases [1] and affecting people aged 85 or older with an incidence of 25–50% [2]. Currently, every 70 seconds one person in America develops dementia, which corresponds to about 450,000 new cases per year. It is estimated that this number will more than double by 2050 [1], mainly due to increased life expectancy.

The exact mechanism(s) underlying AD are subject to enormous research efforts. Until now, approximately 72,000 papers on AD have been published (July 2010, PubMed). About 18% of all actively publishing scien-

tists in neuroscience have published at least one paper in the field [3]. As yet, neither a satisfying therapy nor a preventative cure is available. Furthermore, AD can only be precisely diagnosed postmortem on a neuropathological basis, the so-called Braak stages classify the progress of the disease [4].

AD is one of about 40 identified amyloidoses, which share as their main pathological hallmark the aberrant deposition of endogenous, normally soluble proteins as amyloid fibrils in various tissues. Each of these diseases involves a specific protein and clinical profile, among them Parkinson's disease, the prion diseases, diabetes type II, Huntington's disease, and amyotrophic lateral sclerosis [5]. The major pathological hallmarks in the brain of AD patients are amyloid plaques, consisting predominantly of the Amyloid- β (A β) peptide, and neurofibrillary tangles (NFTs), formed by hyperphosphorylated tau protein. These lesions occur in brain regions involved in learning and memory, i.e. the

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hippocampus, the amygdala, and in the association cortices of the frontal, temporal and parietal lobes. Further A β accumulation is observed in the small blood vessels of the meninges and cerebral cortex, also termed cerebral amyloid angiopathy [6].

The first AD case was described in 1907. Since then, major developments and findings mark the history of AD research in the general context of amyloid-associated disorders. The term amyloid was first introduced by Virchow in 1854 to describe the macroscopic abnormalities associated with clinical symptoms, which appeared to represent the amylaceous constituents of plants upon staining with iodine [7]. Five years later, Friedreich and Kekule suggested that amyloid is a protein rather than starch according to the high nitrogen content [8]. The plaques in the AD brain were first described in 1898 [9], the aniline dye Congo red facilitated specific discrimination from non-amyloid plaques in 1922 [10]. In 1907, Alois Alzheimer's lecture about the first case of the fatal progressive dementia including extracellular plaque and intraneuronal NFT pathology did not receive special attention [11], although as a psychiatrist he was a pioneer at this time by associating pathological changes with dementia symptoms. Alzheimer's colleague, Kraepelin, finally gave the disease its official name in 1910 [12]. Amyloid fibrils from tissue were first visualized by electron microscopy in 1959 [13]. X-ray diffraction studies of isolated fibrils in 1968 revealed the so-called cross- β structure as a common motif [14]. In the 1970s, the availability of amino acid analysis and protein sequencing tools revealed that each amyloidosis is linked to a specific protein [15].

In 1984, A β was identified as the major component of plaques from AD and Down syndrome patients [16, 17]. Tau had already been described in 1975 as an essential protein for microtubule assembly [18], but it was not until one year after the identification of A β that it was identified as the NFT-forming protein [19]. The corresponding MAPT gene was cloned in 1988 [20], again one year after the amyloid- β protein precursor (A β PP) gene containing the A β sequence was cloned from chromosome 21 [21].

AD cases can be categorized into two main categories, the (pseudo-) sporadic late-onset AD (LOAD) and early-onset familial AD (FAD). LOAD is characterized by disease manifestation at ages above 65 years, whereas FAD occurs earlier, sometimes already in the twenties. Increasing age is the major risk factor for LOAD. In addition, the apolipoprotein E (ApoE) gene on chromosome 19 has been demonstrated to represent

a major genetic risk factor. FAD cases likely constitute less than 1% of all AD cases [1]. The usual suspects of FAD are primarily A β PP and presenilin (PS) 1 and 2. A β is generated from A β PP by β - and γ -secretase cleavage. PS resembles the active site of the γ -secretase, which is composed of one PS, nicastrin, APH-1, and PEN-2. In 1991, the first FAD cases were linked to mutations in the A β PP gene [22]. One year later, FAD-causing mutations were mapped to chromosome 14 and in 1995 to chromosome 1 [23], where the genes encoding PS 1 and 2 are localized, respectively. The first transgenic mouse model for AD, which developed plaques caused by A β PP mutation, was presented in 1995 [24]. In 2003, the first A β vaccination trial was eventually performed [25]. However, due to the occurrence of meningoencephalitis in some of the AD patients, this initial trial had to be suspended.

THE AMYLOID HYPOTHESIS

In the early 1990s, the amyloid hypothesis was formulated [26–28]. It proposes that A β deposition represents the central hallmark of AD pathogenesis and states that A β aggregation is the cause rather than an effect of AD, which was initially based on FAD cases and A β toxicity [26–28]. Major evidence came from the analysis of Down syndrome patients, who carry an additional A β PP allele and develop FAD likely because of a dose-dependent effect. Duplication of the A β PP locus was eventually reported to cause FAD in 2006 [29]. Moreover, FAD-associated mutations identified at this time in A β PP were shown to alter its metabolism, leading to increased A β production [28]. A β aggregates are toxic in cell culture and animal models [30] and disaggregation of A β by antibody treatment reverses its toxicity [31]. Arguments against a causative role of tau in AD are that A β aggregates occur earlier than the NFTs [32], tau hyperphosphorylation and aggregation can be triggered by A β aggregation [33, 34], and mutations in tau cause frontotemporal dementia with Parkinsonism, not FAD [35]. A β aggregation was therefore concluded to initiate a neurodegenerative cascade, leading to neuron loss and dementia, which is also termed the amyloid cascade hypothesis [26, 28].

More than 25 years after the identification of A β , the enzymes that generate A β from A β PP, the proteases that clear A β from the brain, as well as other proteins that interact with A β to regulate its abundance, have been characterized. This information provides further support for the amyloid hypothesis [36]. There is, how-

ever, some controversy as to whether A β aggregation exclusively causes AD, which role A β PP, tau, and other proteins play in AD, and how they interact. The toxic key players in the A β aggregation pathway(s) still have to be identified. The amyloid hypothesis implies that A β aggregation is upstream of all obvious pathological events and that inhibition of A β aggregation can prevent AD. So far, a satisfactory therapy based specifically on targeting A β could not be established. However, several promising approaches have now reached the clinic [37].

A β , THE PATHOGENIC KEY PLAYER?

A β aggregation is considered a key event in the pathogenesis of AD [30] as well as sporadic inclusion-body myositis, which is the most common cause of muscle degeneration in elderly people [38]. A β is generated as a normal product of A β PP metabolism via sequential cleavage by β - and γ -secretase, releasing the N- and C-terminal fragments of A β PP, respectively [39,40]. This procedure is called the amyloidogenic pathway of A β PP processing. In an alternative, non-amyloidogenic pathway, A β PP is cleaved by α -secretase between position 15 and 16 of the A β segment, followed by γ -secretase processing, which prevents A β formation [39,40] (Fig. 1).

In the cortex of AD patients and healthy controls, the average A β concentrations of 406 and 221 mg/g were determined, respectively [41]. This extrapolates to several hundred milligrams of A β in a human brain and suggests a non-transient physiological function. Depletion of A β specifically led to neuron death in cell cultures [42]. In mice, A β was shown to have an important role for learning and memory in younger individuals [43]; it may generally regulate cell survival and excitability. Notably, β -secretase knockout mice do not show any deficits, although the A β load is dramatically decreased [44]. After traumatic brain injury (TBI), increased A β , A β PP, and plaque loads are found, which can occur within hours after the initial trauma [45]. Furthermore, there is recent evidence that A β is part of the innate immune system of the brain and functions as an antimicrobiant [46]. It has also recently been reported that the A β content in the interstitial fluid is regulated by the sleep-wake cycle and increases upon sleep deprivation [47].

Several proteases have been identified that clear A β from the brain. Insulin degrading enzyme (IDE) and neprilysin are primarily important for regulation of the

steady-state levels of A β . IDE is a thiol metalloendopeptidase that degrades monomeric A β . Deletion of IDE reduces A β degradation by more than 50% in mice [48]. Neprilysin is a membrane-anchored zinc endopeptidase that degrades A β monomers and oligomers. Depletion of neprilysin causes cerebral accumulation of A β [49]. Further identified proteases for A β degradation are plasmin and cathepsin [50,51], endothelin-converting enzyme [52], and the matrix-metalloproteinase family [53]. Moreover, the A β load is in equilibrium across the blood-brain barrier (BBB) with its efflux mediated by the low-density lipoprotein receptor related protein (LRP1) and its influx mediated by the receptor for advanced glycosylation end products (RAGE) [54]. In the periphery, A β is primarily degraded in the liver and to a lesser extent in the kidneys [55].

In the brain, A β is produced mainly in various cellular compartments of neurons, but has also been detected in glial cells and astrocytes [56]. *In vitro*, “the peptide from hell” [57] rapidly forms amyloid fibrils in aqueous solution and is hard to handle due to its amphipathic character and distinct tendency to aggregate [30]. *In vivo*, A β variants with lengths of 38–43 amino acids, differing in their C-terminus, are produced due to differential cleavage of A β PP by γ -secretase, following a mechanism termed regulated intramembrane proteolysis [58]. The most abundant variants are A β _{1–40} and the more amyloidogenic A β _{1–42}, with an approximate ratio of 10:1 [30]. A β _{1–42} is considered to be a key player in the initiation of AD [30].

FAD-causing mutations flanking the A β segment in A β PP, and in PS 1 and 2 generally modulate A β production and frequently underlie a selective increase in production of A β _{1–42} [59]. About ten FAD-underlying mutations within the A β sequence have been identified (Fig. 1). These variants generally exhibit higher amyloidogenicity and lead to distinct neuropathology [60]. Most of these mutations are localized at positions 21–23, but there are also FAD-related amino acid replacements in the N-terminal segment, such as the Tottori (D7N), English (H6R), and A2V mutation [61, 62]. The identified A β mutations mostly cause autosomal dominant FAD, but two apparently recessive mutations have been reported: A2V [62] and the Japanese mutation E22 Δ [63]. Molecules of the A β population can undergo posttranslational modification, e.g. oxidation of methionine 35 to methionine sulfoxide or N-terminal truncation by aminopeptidases and pyroglutamate formation, i.e. lactam formation of N-terminal glutamate [30,64,65]. Taken together, the A β population in the brain is a heterogeneous peptide pool.

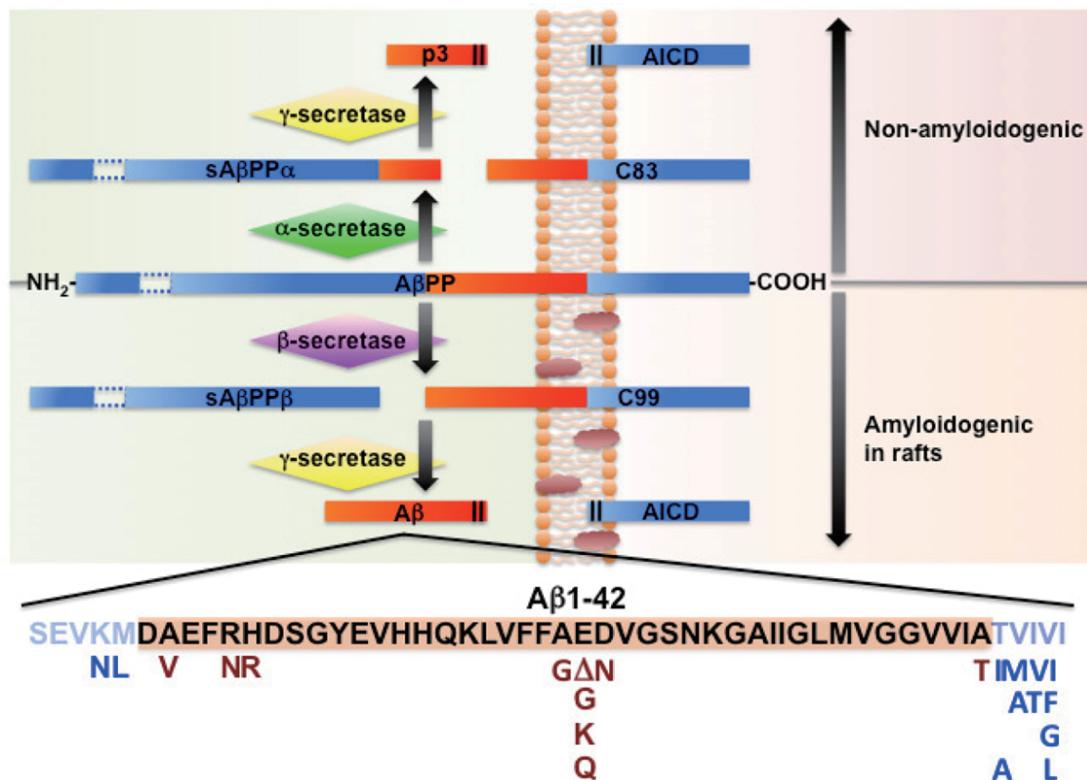


Fig. 1. A β PP processing and A β . A β PP has a length of 695–770 amino acid residues, the variants differing in their N-terminal segment. A β PP can be metabolized in a non-amyloidogenic pathway by sequential α - and γ -secretase cleavage (upper part of the scheme). Processing in the amyloidogenic pathway by sequential β - and γ -secretase occurs in lipid rafts and yields A β (lower part). The sequence of A β_{1-42} is shown, with the identified FAD associated mutations within or flanking the A β sequence indicated below.

A β PP – MORE THAN A SOURCE OF A β

A β PP is produced in a variety of cells throughout our body, with its expression level depending on the respective developmental state. A β PP is found mainly at the plasma membrane, but also in the trans-Golgi network, the endoplasmic reticulum, and at endosomal, lysosomal and mitochondrial membranes [66]. Different isoforms of A β PP with lengths of 695–770 amino acid residues exist. The shortest one – A β PP695 – is the predominant form in the brain. The larger extracellular N-terminal segment consists of several domains, is glycosylated, and constitutes 88% of A β PP695. The small cytoplasmic C-terminal segment comprises the A β PP intracellular domain (AICD). The A β segment includes partly the N-terminal and the transmembrane segment [67]. Assessment of A β PP metabolism has shown that it undergoes rapid turnover in cells [68], with the majority of A β PP molecules being cleaved by α -secretase [39].

The physiological functions of A β PP and its metabolic products are subject to intensive research [67].

A β PP knockout mice are viable but have been shown to display synaptic, learning and memory deficits. The presence of the other APP family members, namely APLP1 and APLP2, has been shown to compensate for the loss of A β PP itself [69]. However, triple A β PP/APLP1/APLP2 knockouts are embryonic lethal [70]. The number of functional synapses generally seems to be modulated by A β PP in a dose-dependent manner [71]. A β PP has been shown to be important for the regulation of neuronal survival, neurite outgrowth, synaptic plasticity, and cell adhesion [72]. It can be phosphorylated by different kinases in the extra- and intracellular segment. Phosphorylation at T668 has received special interest as it leads to altered A β PP processing and A β production [73].

Upon cleavage with α -secretase (Fig. 1), the extracellular sA β PP α is secreted and C83, a C-terminal fragment of 83 amino acid residues also termed A β PP-CTF α , remains at the membrane. Secretion of sA β PP α from presynaptic terminals is triggered by electrical activity and activation of muscarinic acetylcholine receptors. Hence, neuronal activity may increase non-

amyloidogenic processing of A β PP [72]. sA β PP α regulates neuronal excitability and increases synaptic plasticity, leading to enhanced learning and memory. C83 is either degraded in lysosomes [74] or sequentially processed by γ -secretase to AICD/C59 and C-terminal A β PP fragments of approximately 3 kDa termed p3 [40].

Amyloidogenic processing of A β PP (Fig. 1) involves sequential cleavage by β -secretase (also termed BACE1) and γ -secretase. Upon β -secretase cleavage, the extracellular sA β PP β segment is released and the remaining C-terminal C99 fragment can be further processed by γ -secretase, producing A β and AICD, or by caspases to release the neurotoxic peptide C31 [75]. AICD can translocate to the nucleus, where it regulates gene expression and potentially induces production of apoptotic proteins [76].

TAU – A LATECOMER IN AD?

Hyperphosphorylated tau is the major component of NFTs in pyramidal neurons and neuropil threads in distal dendrites in AD. Additionally, tau inclusions are observed in several sporadic disorders, termed tauopathies [35].

The physiological function of tau is to promote the assembly of microtubules and to stabilize them, entailing a role in vesicle transport. Tau has a length of 352–441 amino acid residues and is present in a total of six different isoforms in the human brain [77]. Within three of these isoforms the microtubule-binding domain is encoded by three repeat sequences, whereas the others contain four sequence repeats. The isoforms within these subgroups differ in their N-terminal segments. Phosphorylation and dephosphorylation of tau is catalyzed by various kinases and phosphatases, respectively [78]. Hyperphosphorylated tau spontaneously aggregates into paired helical filaments, which can subsequently form NFTs [79].

Oligomers of tau exhibit cytotoxicity and cause cognitive deficits [80], suggesting a toxic gain of function. Additionally, tau has been described as a prerequisite for A β toxicity [81] and there is evidence that tau aggregation could be seeded in mice by injection of extracted tau aggregates [82]. The number of NFTs correlates with the extent of disease progression in AD, but does not correspond to the neuron loss [83]. Importantly, aggregation of tau also leads to disturbance of axonal transport through its loss of function.

UNDERLYING MECHANISM(S) OF AD

The biological functions of a cell depend on folding of thousands of diverse proteins and hence misfolding underlies the pathology of various diseases. According to the amyloid hypothesis, the key event in the initiation of AD is misfolding and aggregation of A β (Fig. 2). Factors for the misfolding probability of a protein can be intrinsic (based on the amino acid sequence) or extrinsic (based on mostly unidentified external circumstances) [84]. The intrinsic aggregation tendency primarily depends on the charge, hydrophobicity, and on secondary structure propensities [85]. Particularly, high propensity to form β -sheets and low propensity to form α -helices favors amyloid formation [86]. Environmental factors for the misfolding propensity are parameters such as temperature, ionic strength, pH, oxidative stress, macromolecular crowding, and increased concentration of the misfolding protein [85]. For instance, increase in the protein production rate [17] or decrease of protein clearance, or both, can be directly associated with pathogenic misfolding. Misfolded proteins in the cell are either naturally rescued by the cellular quality control machinery or degraded. The latter can be mediated by the proteasome [87], chaperones, and autophagy/lysosomal pathways [88]. The capability of the immune system to recognize and clear protein aggregates requires further investigation. Aggregated A β seems to be a natural target of the immune system. Natural antibodies against oligomeric, fibrillar A β and plaques have been identified and their abundance shown to decrease with increasing age [89, 90]. These findings suggest that reduced efficiency of the immune system could lead to decreased A β plaque clearance.

Amyloid formation is not necessarily an undesired event. In fact, functional amyloid structures exist in many living organisms for manifold specific purposes, e.g. in spider silk [85]. The highly ordered fibril structure carries information, which self-propagates the morphology by seeding and cooperative binding of further subunits. Amyloid fibrils share their cross- β structure with β -sheets aligned perpendicular to the fibril axis. Diverse morphologies, however, can even be formed by the same protein, such as A β_{1-40} [91]. It has been proposed that all proteins can form amyloid fibrils under appropriate conditions. As in a simple polymer, stability of the β -sheets of the fibril is built by hydrogen bonds between the β -strands, but not by specific interactions between amino acid residues [84]. Deciphering

the "amyloome" should reveal, which proteins can form amyloids under naturally occurring conditions [92].

The only accepted genetic risk factor for sporadic AD identified so far is the ApoE4 allele, of which one and two copies lead to 3–4 times and 15–19 times increased risk, respectively [93]. In addition, ApoE4 lowers the age of onset by about ten years per allele [94]. Although the exact mode of action is unclear, ApoE4 binds to A β and seems to trigger A β and tau aggregation [95]. Recently, three further genes have been reported to represent risk factors for LOAD: CLU/APOJ, PICALM, and CR1 [96,97].

Extrinsic risk factors for AD include high cholesterol, diabetes mellitus, a low educational level, TBI, consumption of a high calorie diet, sedentary lifestyle, cardiovascular disease and risk factors thereof, and reduced cognitive reserve capacity of the brain [98]. The mechanisms are unclear. It is known that cholesterol is essential in neuronal membranes and concentrated in lipid rafts, where the β - and γ -secretase are assembled and A β is generated [99]. ApoE is the main cholesterol transporter in the brain, whereby ApoE4 has the lowest efficiency in maintaining healthy lipid turnover in the membranes [100]. High cholesterol increases A β -associated pathology in transgenic mice, whereas cholesterol-lowering drugs decrease it [101] and also cause a lower incidence of AD in humans [102].

The various A β variants in the brain could follow different assembly pathways, with different relevance in the initiation of AD. The 20 so far identified FAD-causing A β PP mutations flanking the A β segment generally lead to increased or modified A β production. Over 150 mutations in PS 1 and about 20 in its homologue PS 2 have been identified to mainly cause FAD by increasing the production of A β_{1-42} [103] (see <http://www.alzforum.org/> and <http://www.molgen.ua.ac.be/ADMutations/default.cfm?MT=0&ML=0&Page=Home> for AD-associated mutations). There is evidence that A β_{1-40} plays a protective role rather than being a key player in the pathogenesis of AD, most likely by exhibiting low amyloidogenicity and the potential to inhibit aggregation of other A β variants [104]. Vascular A β deposits, however, contain mainly A β_{1-40} [105]. It has also been suggested from *in vitro* experiments that pyroglutamate A β seeds pathogenic aggregation *in vivo*. Inhibition of the glutaminylcyclase - the enzyme that catalyzes N-terminal pyroglutamate formation – inhibits A β aggregation and toxicity in rodents [65].

The ordered misfolding pathways of different proteins seem to be similar, proceeding from (partially)

unfolded monomers to oligomers and finally to fibrils that accumulate. This process follows the mechanism of a nucleated polymerization [30,106], involving an initial lag phase followed by a phase of seeded aggregation, which is in agreement with typical A β aggregation kinetics [107]. The lag phase, as in crystallization processes, can be eliminated or shortened by seeding, explaining the transmission propensities of prion-related misfolding diseases. Recently, a mathematical model involving the possibility of fibril breakage associated with acceleration of the aggregation reaction was described [108].

An important question to address is whether A β exhibits the strain phenomenon observed for prions. Prions form aggregates with differences in structure, transmissibility, and protease resistance. These strains are propagated by seeding of monomeric populations of molecules [109]. Transmission of amyloidoses in the classical way was only shown for prion diseases. AD and other amyloidoses may be transmissible as well, as shown by inoculation of brains [110] and cell to cell transmission [111].

The elucidation of the pathogenic mechanisms in AD is challenging. Amyloid formation by a peptide or protein immediately results in loss of function. If the aggregates exhibit toxic effects this event can additionally be considered a toxic gain of function, which can also be caused by modified function, e.g. missense mutations in PS shift the cleavage of A β PP to residue 42 of A β , thereby favoring aggregation of A β [58,112]. AD involves a huge complexity of pathological processes and protein interactions, which result in manifold disturbances of the cellular function. Initially, A β plaques were considered the pathogenic species in AD. However, accumulating evidence suggests that they could represent final waste deposits, with the oligomeric intermediates representing the key toxic players [58]. In support of this concept, oligomeric species have been shown to be elevated in AD and correlate with cognitive dysfunction [113]. Plaques could, however, provide a source for soluble toxic species [58].

Oligomers of various proteins involved in amyloidoses have been shown to exhibit toxic characteristics and could therefore be generic toxins [114]. Numerous studies on toxic A β oligomers identified dimers [115], pentamers [116], ADDLs, pores/annular oligomers, A β^{*56} (56 kDa), globulomers, and others [117]. The exact aggregation pathway(s) and the mechanisms of oligomer toxicity, their relevance, and whether these oligomers are on- or off-pathway intermediates remains to be elucidated.

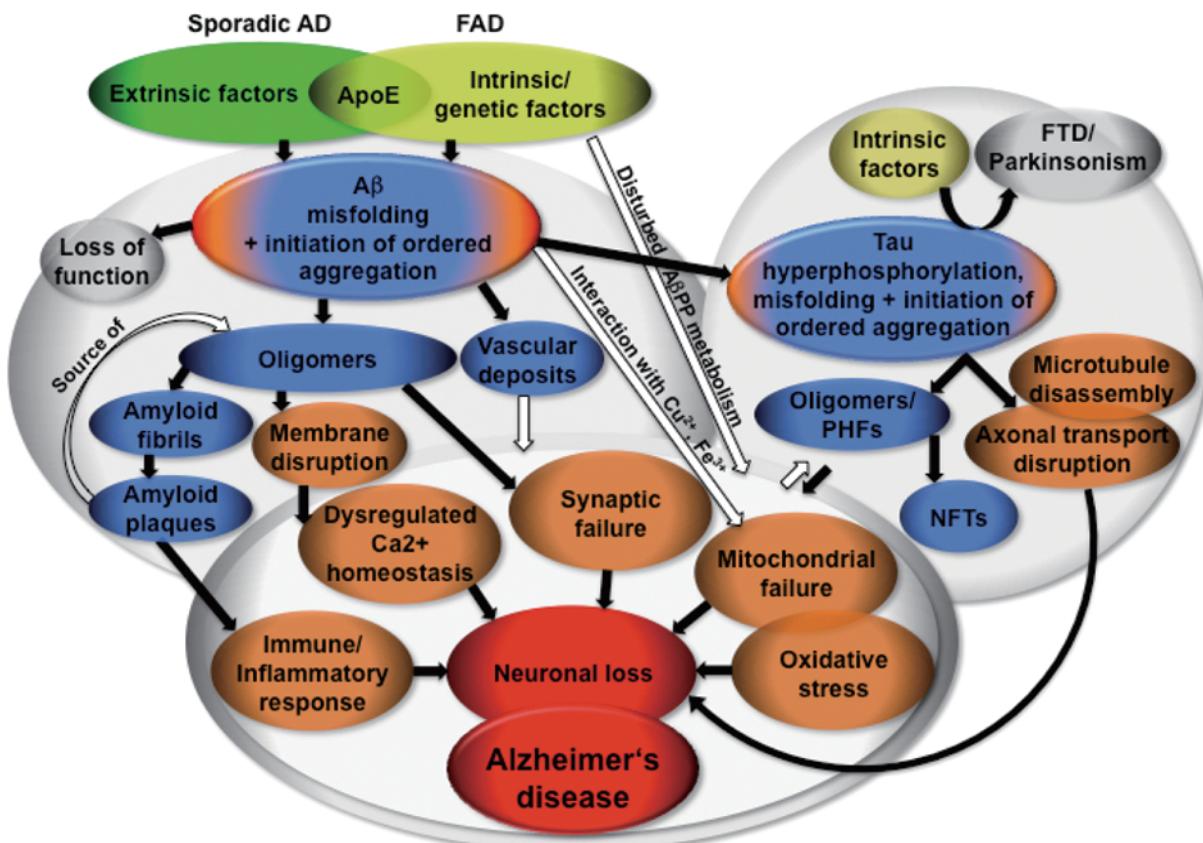


Fig. 2. AD is a complex neurodegenerative disease. A β aggregation is considered the key event in the initiation of AD and can be caused by intrinsic/genetic factors (e.g. traumatic brain injury, neural reserve, vascular/heart disease, lifestyle) or extrinsic factors (e.g. A β PP, PS1 or 2, degradation/rescue machinery), shown in yellow and green/black font in white ovals. The former are associated with FAD, whereas the latter rather cause sporadic AD. A β aggregation can trigger aberrant tau phosphorylation and aggregation. Aggregation of tau leads to loss of function, which causes axonal transport disruption by microtubule disassembly. The intermediates and products of the ordered aggregation mechanisms (blue/black font in grey ovals), with A β oligomers as key players causing various potential pathological and toxic events (indicated in orange/white font in grey ovals), which eventually lead to neuronal loss, presenting as dementia (red/white font in black ovals).

The proposed neurotoxic effects of A β oligomers are synaptic failure, membrane permeabilization, mitochondrial failure and oxidative stress, recruitment of cellular factors or activation of cellular processes such as apoptosis and inflammation [78] (Fig. 2).

Oligomeric A β is synaptotoxic, which could lead to subsequent death of neurons [118,119]. In AD, the number of synapses is significantly reduced and synapse reduction correlates better with cognitive deficits than plaque load [120]. Several mechanisms for the disruption of synapse function have been described. Oligomeric A β , for example, has been reported to disrupt phosphatidylinositol metabolism [121] and glutamate uptake [113]. A β aggregation in AD has moreover been associated with specific disturbance of the activity of the default network [122].

Membrane disruption by A β oligomers is a likely pathogenic mechanism. The channel theory, or amyloid pore hypothesis, states that A β forms channels or pores in the cellular membrane, which render them more or less specifically permeable for ions such as Ca $^{2+}$, and lead to disturbed ion homeostasis, resulting in apoptosis [123]. The disturbance of Ca $^{2+}$ homeostasis has been reported to be a hallmark of AD [124], and can be caused by A β oligomers. Pores were observed in samples from AD patients by high resolution transmission electron microscopy [125], and *in vitro* by atomic force microscopy [126]. Further support for these findings is provided by a study showing that inhibitors of predicted A β Ca $^{2+}$ channels reduced neurotoxicity of A β *in vitro* [127]. There is also evidence that A β increases Ca $^{2+}$ influx through receptor targeting

and that A β aggregation can be triggered by increased Ca $^{2+}$ [128].

20% of the body's total basal oxygen consumption occurs in the brain, which makes it particularly prone to the generation of oxidative stress, resulting from mitochondrial dysfunction [129]. Reactive oxygen species can damage a variety of the biomolecules in the cells, such as DNA/RNA, lipids, and proteins. Increased oxidative stress is described as a common phenomenon in AD [130]. There is evidence for oxidative DNA damage [131] and lipid peroxidation in the presence of plaques in transgenic mice and in AD brain [53,132]. Many proteins are found to be excessively oxidized in AD, which could result in neuronal death upon loss of function [130]. There is already evidence for increased oxidative stress in individuals with mild cognitive impairment (MCI), a transitional stage to AD from which about 10–15% of patients convert per year [133], suggesting a rather early role in the development of AD [130].

Post-mortem studies of AD brains revealed altered mitochondrial enzyme activities, morphology, and reduced numbers of mitochondria, even before NFTs occurred [53,134]. Moreover, A β has been shown to accumulate in structurally disrupted mitochondria in AD [134], to be toxic to mitochondria, and to disrupt their function at the synapse [135], leading to disturbed energy metabolism, generation of reactive oxygen species, and ultimately to apoptosis.

Human A β may play a role in the generation of reactive oxygen species. Three histidine residues at positions 6, 13, and 14 of the A β sequence can chelate Cu $^{2+}$ and Fe $^{3+}$ ions, which can be reduced by oxidation of methionine 35, leading to the generation of reactive oxygen species. The data on the role of oxidized methionine 35 for A β toxicity are contradictory. Clinical trials with antioxidative substances and metal chelators gave inconsistent results [136]. Taken together, the general consequences of increased oxidative stress, potentially mediated by A β seem to have a more pronounced impact on the AD pathology than the oxidation of A β itself.

The role of intracellular tau aggregation and its localization within or beside the pathological cascade needs further clarification. Similar to the findings with A β oligomers and plaques, there is evidence that tau oligomers rather than the NFTs act as the neurotoxic species [137].

It is under investigation whether intracellular A β aggregation is the key event in the pathogenesis of AD. Intracellular A β has been detected in nerve tissue of

AD patients and healthy individuals since the eighties [138]. There is evidence from human brain tissue and mouse models that intracellular A β accumulation, particularly of A β_{1-42} , precedes extracellular A β aggregation and correlates better with the appearance of disease symptoms in mice [139,140]. In inclusion body myositis, A β exclusively accumulates intracellularly [66].

The toxic mechanisms are as unclear as the assembly states of intracellular A β , but hypothetic pathways involve the damage of cellular structures and activation of apoptotic pathways [56,66]. A β can be generated in early endosomes and secreted in association with exosomes. Exosomal proteins accumulate in plaques, which could indicate an intracellular basis for a pathogenic A β aggregation mechanism. Lipid rafts could serve as a seed production site for A β aggregation [141,142]. Moreover, A β uptake by interaction with various receptor proteins and potential intracellular seed formation has been described [143].

A further explanation for the cognitive deficits in AD is the loss of cholinergic neurons. This results in reduced levels of acetylcholine, which has been ascribed to the neurotoxic effect of A β and is also observed for some anesthetics targeting acetylcholine-sensitive cells [144]. Furthermore, inflammation in AD brains causes elevated markers for activated microglia and reactive astrocytes, which surround amyloid plaques [145], and led to consideration of anti-inflammatory drugs as AD therapeutics.

Surgery under anesthesia can cause transient reversible AD-like symptoms in elderly patients and after prolonged anesthesia, which is termed postoperative cognitive dysfunction (POCD) [146]. *In vitro* studies aimed at elucidating the involvement of AD mechanisms showed that several anesthetics – particularly the small-sized inhaled anesthetics isoflurane and halothane – could promote A β aggregation. In contrast, the interaction of A β with the bulkier injected anesthetics such as thiopental and diazepam seems to be sterically hindered [146–149]. Isoflurane and halothane were shown to increase the plaque load in a murine AD model [150], and isoflurane has recently been shown to accelerate neurofibrillar pathology in a murine tauopathy model [151]. These findings point to the importance of further investigation of cognitive dysfunction after anesthesia or reconsideration of anesthetic protocols for patients with increased AD risk, (i.e. due to increased age, TBI, or genetic predisposition).

In summary, AD is a very complex disease. The manifold pathomechanisms and pathologic observa-

tions remain to be classified and arranged into an unequivocal cascadic sequence. The main processes identified are accumulation of A β and oligomer formation, ultimately leading to plaque pathology, synaptotoxicity, disturbance in ion homeostasis, mitochondrial toxicity and oxidative stress, disturbed signal cascades and metabolism, tau hyperphosphorylation and NFT formation, inflammatory responses, neurotoxicity, and complex neuronal dysfunction. As a result of such a complex cascade of events the final disease presentation is one of clinical dementia.

CHALLENGES ON THE ROAD TOWARDS THERAPEUTIC APPROACHES

Major roads, which have so far been followed for the development of therapeutic approaches, include the intervention in A β generation and aggregation as well as the prevention of neuronal loss. The long list of current and future challenges include the ability to bridge the *in vitro-in vivo* gap, i.e. to appraise the relevance of findings from *in vitro* studies, to reproduce these findings in animal studies and finally also in clinical trials [152]. Reproducibility of *in vitro* studies with A β is challenging to obtain since proper handling and the quality of the applied material are crucial [107]. Variation of the fibrillization conditions *in vitro* leads to fibril polymorphism [91], which challenges the *in vivo* relevance of these studies. *In vitro* preparations, however, proved to be toxic in transgenic mice [107]. The peptide concentration in *in vitro* experiments usually exceeds the physiological condition, but local concentrations *in vivo* could be considerably higher as well [143].

The role of the various A β variants found *in vivo* needs further elucidation to separate the guilty parties from the innocent bystanders. The study of potential co-aggregation and cross seeding of the different natural A β variants may reveal the existence of fibril strains. It will also be important to investigate whether intra- or extracellular A β causes the major risk.

It is challenging to detect and identify the transient oligomers in the pathway(s) of A β aggregation. Cross-linking of aggregating A β yielded ambiguous results [153]. Enzyme-linked immunosorbent assays were found to detect oligomers inefficiently [154] and application of different antibodies, combined with the uncertainty of the detected oligomeric state challenge their reproducibility. So far, oligomeric intermediates have been separated via Western blotting, where oligomer formation can be caused by sodium dode-

cyl sulfate added to the samples [155]. Application of further methods for the structural characterization of prefibrillar intermediates is, however, emerging with increasing success, e.g. nuclear magnetic resonance [156].

The inhibition of A β generation is promising but also challenging in different aspects. Inhibition of the β -secretase is technically challenging as the active site is large and inhibitors have to be sufficiently small to cross the BBB [157]. Moreover, inhibition of β - and γ -secretase leads to severe side effects due to the existence of various substrates other than A β PP [39]. An activator of α -secretase to favor the non-amyloidogenic pathway of A β PP processing will be challenging to develop, which also applies for the activation of the A β degrading enzymes. Overexpression of both IDE and neprilysin has, however, been reported to prevent plaque formation in mice [158].

Animal models do not normally exhibit all aspects and forms of the disease. For example, A β PP based transgenic mouse models do not include NFTs and many of them are based on rare FAD forms. Furthermore, murine A β is present in addition to the transgenic human A β PP variants in these models and could hence interfere with the aggregation process. Despite this setback, many of the pathological features of AD could nevertheless be included in mouse models over the years. In fact, a variety of different animal models are now available, enabling various applications, including zebrafish, *C.elegans*, *drosophila*, and yeast [159].

Active or passive vaccination against A β seems to be the most promising therapeutic approach to date. The modes of action involve clearance of plaques, inhibition of aggregation, and causation of a peripheral sink to clear A β from the brain. To date, at least 13 A β immunotherapies are in clinical trials [160]. Antibodies binding to A β have the risk of targeting A β PP as well. Thus, the development of conformation-specific antibodies may be promising, with the added advantage that they could also serve in diagnostics.

A β aggregation had been suggested to take place several decades before the first AD symptoms occur. In 2008, it was shown that plaques can be formed within 24 hours [161], which provides space for previous causes of A β -associated AD. Moreover, cognitively normal aged individuals with pronounced plaque load were identified [162], which could indicate a non-toxic pathway of plaque formation. Clearance of plaque burden does not necessarily lead to improvement of cognitive performance and expansion of life span [163]. Consequently, vaccination and other therapies may only be efficient before plaque formation.

Antemortem diagnostic tools are limited, but promising imaging techniques for the diagnosis of AD have been described. Imaging of the plaque load with Pittsburgh compound B (PiB) proved to be useful, although the binding capacity for different fibril morphologies could differ [164]. Furthermore, positron emission tomography represents a promising tool for monitoring the disease stage [165]. Functional magnetic resonance imaging (fMRI) [166] seems to be promising for early and specific diagnosis of AD by measuring the brain volume and prediction of conversion from MCI to AD. Diagnosis via biochemical analysis of cerebrospinal fluid (CSF), involving the analysis of the distinct A β variants, oligomeric A β , increased phosphorylation and tau load are promising, although there is some controversy [167].

Finally, it is important to carefully choose (control-) patients for clinical trials, as it is not yet clear how many different forms of AD exist and heterogeneity in the subject population complicates the evaluation.

CONCLUSION

AD is a complex disease and represents a tremendous problem in our aging populations, where the major aim is to cure and prevent AD. Collaboration of experts with different backgrounds will be required to further elucidate the molecular mechanism(s) and interactions of different pathological aspects of AD. It remains to be elucidated to which extent the different A β fractions and tau contribute to AD, how many forms of the fatal progressive dementia exist, and how and when to interfere efficiently. The emergence of early and precise diagnosis is of paramount importance for a more specific and opportune intervention in AD.

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