HHS Public Access

Author manuscript

Neurochem Int. Author manuscript; available in PMC 2018 November 01.

Published in final edited form as:

Neurochem Int. 2017 November ; 110: 1–13. doi:10.1016/j.neuint.2017.08.007.

APP/Aβ Structural Diversity and Alzheimer's Disease Pathogenesis

Alex E. Roher[1,2], **Tyler A. Kokjohn**[3], **Steven G. Clarke**[4], **Michael R. Sierks**[5], **Chera L. Maarouf**[6], **Geidy E. Serrano**[6], **Marwan S. Sabbagh**[7], and **Thomas G. Beach**[6]

[1]Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013

[2]Division of Clinical Education, Midwestern University, Glendale, AZ 85308

[3]Department of Microbiology, Midwestern University, Glendale, AZ 85308

[4]Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, Los Angeles CA 90095-1569

[5]Department of Chemical Engineering, Arizona State University, Tempe, AZ 85287-6106

[6]Laboratory of Neuropathology, Banner Sun Health Research Institute, Sun City, AZ 85351

[7]Alzheimer's and Memory Disorders Division, Barrow Neurological Institute, Phoenix, AZ 85013

Abstract

The amyloid cascade hypothesis of Alzheimer's disease (AD) proposes amyloid-β (Aβ) is a chief pathological element of dementia. AD therapies have targeted monomeric and oligomeric Aβ 1-40 and 1-42 peptides. However, alternative APP proteolytic processing produces a complex roster of Aβ species. In addition, Aβ peptides are subject to extensive posttranslational modification (PTM). We propose that amplified production of some APP/Aβ species, perhaps exacerbated by differential gene expression and reduced peptide degradation, creates a diverse spectrum of modified species which disrupt brain homeostasis and accelerate AD neurodegeneration. We surveyed the literature to catalog Aβ PTM including species with isoAsp at positions 7 and 23 which may phenocopy the Tottori and Iowa Aβ mutations that result in early onset AD. We speculate that accumulation of these alterations induce changes in secondary and tertiary structure of Aβ that favor increased toxicity, and seeding and propagation in sporadic AD. Additionally, amyloid-β peptides with a pyroglutamate modification at position 3 and oxidation of Met35 make up a substantial portion of sporadic AD amyloid deposits. The intrinsic physical properties of these species, including resistance to degradation, an enhanced aggregation rate, increased neurotoxicity, and association with behavioral deficits, suggest their emergence is linked to dementia. The generation of specific 3D-molecular conformations of Aβ impart unique

Corresponding Author: Alex E. Roher, MD., PhD., Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013, Division of Clinical Education, Midwestern University, Glendale, AZ 85308, Phone: 480-229-6667, aeroher@gmail.com.

Competing Interests

Roher AE, Clarke SG, Kokjohn TA, Maarouf CL, Sierks MR and Serrano G, have no conflicts of interest to declare.

biophysical properties and a capacity to seed the prion-like global transmission of amyloid through the brain. The accumulation of rogue Aβ ultimately contributes to the destruction of vascular walls, neurons and glial cells culminating in dementia. A systematic examination of Aβ PTM and the analysis of the toxicity that they induced may help create essential biomarkers to more precisely stage AD pathology, design countermeasures and gauge the impacts of interventions.

Introduction

Alzheimer's disease (AD) is characterized by the deposition of amyloid plaques and neurofibrillary tangles (NFT) in the brain. The main component of extracellular amyloid plaques is the amyloid-β peptide (Aβ), an approximately 4 kDa fragment derived from the larger amyloid precursor protein (APP) by the concerted action of β- and γ-secretases [1]. The Aβ peptides polymerize into insoluble ~10 nm filaments which accumulate in senile plaques and the walls of cerebral blood vessels. The NFT are aberrant aggregates mainly composed of tau, a phosphorylated microtubule-associated protein that aggregates into insoluble intraneuronal paired helical filaments [2]. While recognizing the importance of NFT as potential co-pathogenic species in AD, in this critical review we focus specifically on the role of Aβ.

The evolutionary conservation of Aβ suggests this molecule has an adaptive value and important function(s) in the maintenance of CNS homeostasis. Of all 30 mammalian orders, which began to diverge about 90 million years ago, rodents are the only known species harboring amino acid substitutions deviating from the ancestral Aβ sequence. In sharp contrast with humans and many other mammals, age-associated amyloid deposits do not accumulate in rodents (with the exception of the brush tailed rat) *in vivo* [3,4], even though synthetic rodent Aβ peptides produce congophilic filaments *in vitro* [5,6]. Animal and cellular models are necessary for ascertaining disease mechanisms and promoting drug discovery efforts. However, there are still considerable challenges in translating scientific findings from these models into effective clinical interventions.

The amyloid cascade hypothesis is currently the most widely accepted general theory to explain the pathophysiology and clinical evolution of AD. The hypothesis posits Aβ40 and Aβ42 peptides are the critical elements in AD pathogenesis, through their intra- or extracellular neuropil and vascular accumulation. Notwithstanding the genetic evidence suggesting a crucial role for Aβ, considerable controversy still exists over the precise role(s) of amyloid in AD pathogenesis and pathophysiology [7–9]. Amyloid plaques correlate weakly with the clinical progression of AD and are preceded by tau neurodegeneration and brain atrophy in limbic brain regions [10–19]. To account for discrepancies between amyloid deposition and AD dementia some investigators suggest that soluble oligomeric Aβ are the most toxic species. The literature pertaining to the role of oligomeric Aβ in the pathogenesis and pathophysiology of AD is extensive [20–23] with almost 5,000 articles listed under "oligomeric A-beta" in PubMed. Excellent reviews on these topics can be found in references [1,22,25–28] However, no consensus exists regarding the molecular form(s) of Aβ ultimately responsible for the neurological decline associated with AD, the form(s) which should be therapeutically targeted or the optimal time to commence treatment. The

timing of the initial Aβ accumulation and its propagation during the course of disease remains controversial [24]. Likewise, whether Aβ accumulation in the CNS is influenced by Aβ pools originating from peripheral tissues and/or the systemic circulation is unclear [25–28].

The hallmark of AD amyloid found in demented subjects is its immense complexity. Commonly presumed to be composed of Aβ40 and Aβ42 species, extensive posttranslational modifications (PTM) produce a wide array of molecules differing in physical size and chemical/conformation properties. Analogous to the situation observed with other proteinopathies, some of these potentially toxic modified Aβ conformers may promote the proliferation of highly organized amyloid filaments [29–31].

We hypothesize that in late onset AD (LOAD), specific Aβ-related species with shorter or longer sequences and/or altered by PTM enhance noxious amyloid deposition and neurotoxicity. Based on these assumptions, we review experimental evidence revealing the physicochemical nature of potentially neurotoxic amyloid species linked to AD. We consider neglected factors such as covalent modifications of Aβ and its aggregation states that may influence AD pathophysiology and have important implications for the design of immunotherapies. We consider APP proteolysis fragments and peripheral Aβ sources as potential factors influencing neurodegeneration and cognitive dysfunction. In addition, we propose tactics to aid the search for prospective Aβ biomarkers and therapeutic targets.

Amyloid-β posttranslational modifications and AD pathophysiology

Structural alterations in the peptide backbone of Aβ could account for the differential deposition and stability of these molecules in AD [32]. Detailed analyses have revealed that the species present in AD brains are modified extensively [33]. Furthermore, the Aβ peptides isolated from amyloid plaque cores possess a heterogeneous array of N- and C-termini and variable quantities of water soluble and water insoluble Aβ [32,34]. The fundamental chemical characteristics of the Aβ polypeptides are dictated by the amphipathic nature of these molecules, the presence of non-polar and polar domains and an abundance of charged amino acid residues which impose a diverse array of secondary and tertiary structures. Amyloid-β peptides ending in residues 38 to 49, a part of the transmembrane domain of the APP molecule, are progressively more hydrophobic due to the enrichment of non-polar amino acids which decrease solubility and increase aggregation propensity. The removal of charged amino acid residues at the N-terminal region of Aβ by aminopeptidases, endopeptidases or modification by glutaminyl cyclase will also have critical consequences for the intermolecular ionic interactions of the Aβ peptides since this region contains Asp and Glu at positions 1, 3, 7 and 11, and Arg, Lys and His at positions 5, 6, 13, 14 and 16. Deletions or additions in the Aβ sequence will result in differences in molecular folding patterns and intermolecular reactivity. The central domain of Aβ from Leu17 to Lys28 also contains a conserved hydrophobic domain (Leu17-Val18-Phe19-Phe20-Ala21) and the negatively charged residues Glu22 and Asp23. In the following section we give an account of the most important PTM present in the Aβ peptides.

Aspartyl isomerization

Aspartic acid and asparagine residues are particularly subject to non-enzymatic modification reactions that covalently alter the structure of the polypeptide chain. The proximity of the side chain carbonyl group of Asp/Asn to the adjacent residue amide nitrogen induces the formation of a five-membered succinimide ring intermediate [35] which is subject to enhanced racemization [36]. Spontaneous hydrolysis of the L- and D-succinimide intermediates generate a mixture of L- and D-aspartyl and L- and D-isoaspartyl residues [35]. The presence of the isoaspartyl residue distorts the peptide chain to give a kinked polypeptide conformation that resembles a C-terminal substituted Asn residue. Racemization may also occur via radical reactions [37]. L-isoaspartyl residues (and to a lesser extent D-aspartyl residues) can be recognized intracellularly by the protein L-IsoAspartyl (D-aspartyl) O-methyltransferase (PIMT) which initiates their conversion to L-aspartyl and D-isoaspartyl residues [38]. Tryptic digestion and reverse-phase HPLC separation of AD Aβ peptides yielded several isoforms comprising residues Aβ1-5 and Aβ6-16 [32]. Amino acid composition, amino acid sequence analysis, mass spectrometry, enzymatic methylation and stereoisomer determinations demonstrated structural rearrangements of Asp residues at positions Aβ1 and Aβ7. L-isoAsp was the predominant form with D-isoAsp, L-Asp and D-Asp present as minor components, as would be expected for succinimide-mediated degradation. Approximately 75% of the Aβ peptides in the AD brain parenchymal amyloid plaque cores contain isoAsp at position Aβ7 with the amount of isoAsp at position Aβ1 more difficult to estimate due to the variable degree of N-terminal degradation. A third Aβ isoAsp site at position 23 has been reported to accelerate the *in vitro* aggregation kinetics of synthetic Aβ1-42 [39–41]. Interestingly, the Aβ mutation at position 23 Asp Asn (Iowa) produces heavy vascular amyloidosis associated with dementia and intracerebral hemorrhages. In this form of familial AD, an isoAsp at position 23 is produced by deamidation of the mutant Asn residue to Asp followed by isomerization, again via a succinimide intermediate [42–44]. The structural resemblance of isoAsp and Asn residues described above may provide some insight into the pathology associated with the Aβ23 Iowa mutation. Another Aβ mutation reported at position Aβ7 Asp Asn (Tottori) alters the conformational dynamics of Aβ, accelerates the rate of oligomerization and affects metal interactions [45–48].

While immunohistochemical studies suggest that the isoAsp at position 23 is mainly associated with the vascular amyloid deposits, the isoAsp at position 7 appears to be abundant in both parenchymal plaque and vascular related amyloid [43,44,49]. These studies also confirmed that in AD subjects the Asp residues at position 1, 7 and 23 are partially isomerized. The preferential localization of isoAsp at position 23 in vascular deposits of Aβ suggests the isomerization event occurs prior to its vascular deposition, soon after Aβ formation. Alternatively, the physicochemical conditions in the vascular compartment may favor the isoAsp23 modification. Conversion of Asp23 to isoAsp alter the kinetics of polymerization and may promote propagation of amyloid in the AD brain [42]. Recent cryo-electron microscopy (cryo-EM) observations permitted the 3D-structural reconstruction of the Aβ42 amyloid filaments [50]. The model predicts that the negatively charged C_β carboxyl group of Asp23 hinders a more advantageous packing in the stacking of Aβ42 dimer interfaces. Decreasing electrostatic repulsion between adjacent Asp residues will

result in a more stable filamentous structure. The formation of IsoAsp may mimic the Asn23 Iowa mutation by displacing the C_β side chain carboxylate to the $23C_\alpha$.

We propose that Aβ isoAsp at positions 7 and 23 in the AD brain may induce conformational changes analogous to the Tottori and Iowa Aβ mutations which are localized at the same positions of the Aβ peptide and associated with early onset AD. These alterations cause changes in secondary and tertiary structure of the Aβ that may facilitate toxicity, seeding and propagation, perhaps by serving as templates converting unmodified Aβ species into self-transmissible amyloid species in vitro. It has been reported that reversion of isoAsp into Asp occurs in Aβ in the presence of PIMT and the methyl donor S-adenosyl methionine, resulting in the partial blockade of Aβ fibrillogenesis [51]. IsoAsp PTM are undetectable by routine mass spectrometry, since the Aβ peptides with IsoAsp alterations have an atomic mass identical to native Aβ-containing Asp residues. However, estimation of isoAsp can be performed by the enzymatic methods published by Dai et al. [57], Tomidokoro et al. [44] or by electron capture dissociation combined with Fourier transform mass spectrometry [52]. In addition, using a combination of HPLC and mass spectrometry, it is possible to simultaneously determine both racemization and isomerization in Aβ [53]. The conformational changes induced by Aβ PTM, alone or in combination, could also mimic the stereochemical disturbances elicited by known deleterious familial AD amino acid substitutions such as Ala21→Gly (Flemish), Glu22→Gln (Dutch), Glu22→Gly (Artic), Glu22→Lys (Italian), in addition to the Asp23→Asn (Iowa) and Asp7→Asn (Tottori), mutations described above. The transition of the peptide bonds from C_α-C_α to C_β-C_α carbons, drastically reorients the carboxylate and amino groups which alters the conformation of Aβ peptides and their isoelectric points. This facilitates the generation of β-pleated sheets [54–57] thereby rendering these molecules more stable and resistant to enzymatic degradation [58,59]. Interestingly, while the isoAsp at position Aβ1 blocks BACE-1 β-secretase hydrolysis, cathepsin B activity efficiently hydrolyzes peptides with isoAsp at this position [58]. Additionally, it has been reported that a membrane bound β-secretase can cleave in the presence of a D-Asp residue [60]. IsoAsp modifications disrupt the ordered assembly of the α-helix by affecting the stability of the intra- and inter-molecular interactions such as hydrogen bonding, salt bridges and hydrophobic interactions, in turn accelerating rates of Aβ oligomerization and fibril formation [42,44,47]. These observations strengthen the contention that Aβ isoAsp isomerization is a potential triggering mechanism for AD amyloidosis and Aβ neurotoxicity.

Pyroglutamate modification

Amyloid-β species containing pyroglutamate at position 3 (Aβ3pE) have been identified in parenchymal plaques, vascular deposits [61,62], presynaptic sites [63] and lysosomes [64]. About 50% of the Aβ peptides present in purified amyloid plaque cores and about 11% of the total Aβ mass in isolated vascular amyloid deposits have N-terminal Aβ3pE [65]. The formation of Aβ3pE requires the removal of the first two N-terminal Aβ amino acid residues followed by the action of the enzyme glutaminyl cyclase [66]. Numerous investigations have revealed the presence of this peptide in Aβ deposits, its intrinsic physical properties such as resistance to degradation, fast aggregation rate, increased neurotoxicity, association with behavioral deficits, capacity to form hybrids with other Aβ species as well as its potential

role in AD pathogenesis [66–87]. Antibodies against the Aβ3pE modified peptide tested in transgenic (Tg) mouse models decreased Aβ deposits, inhibited Aβ aggregation and reduced behavioral dysfunction [88–90]. It has been proposed that the Aβ3pE peptide could be a potential seeding template of highly neurotoxic Aβ [70,82,91]. Of the many Aβ PTM, only one, Aβ3pE, has been targeted by immunotherapy and is currently in phase-1 clinical testing by Eli Lilly. Unfortunately, this antibody apparently evoked an undesirable immunogenic response in immunized individuals (see: Fagan T. Alzforum News, AAIC-Toronto, 2016, August 24, 2016).

Phosphorylation

Phosphorylation of Aβ at Ser8 by protein kinase A [92,93] enhances aggregation and toxicity. Phosphorylation of Aβ at Ser26 by human cyclin-dependent kinase-1 has also been reported to increase Aβ toxicity [94,95]. It is possible that Ser phosphorylation has been overlooked because the often employed solubilization process utilizes formic acid which readily hydrolyzes esterified phosphate groups. In addition, several studies have suggested that in the AD brain Aβ L-Ser26 can be converted to D-Ser. This racemization apparently produces toxic Aβ fragments that may play a role in neurodegeneration [96–98].

Oxidation

Oxidation of Aβ at Met35 to sulfoxide (S=O) and sulfone (O=S=O) forms has been the object of intense examination. In AD and mild cognitive impairment, oxidative stress mediated by free radicals instigate protein oxidation, lipid peroxidation and reactive oxygen species (ROS) production conducive to synaptic damage with neuronal and glial demise [99]. Met35 appears to regulate copper-catalyzed oxidation and aid in the generation of noxious hydrogen peroxide [100]. Electron spin resonance studies have confirmed that Met35 intervenes in free radical production. Substitution of Met35 with Val or Leu residues eliminates free radical production, oxidative stress and hippocampal toxicity of Aβ [99,101,102]. Furthermore, induction of Met-sulfoxide reductase in Tg mouse models protected neurons from Aβ toxicity [103]. Circular dichroism, thioflavine-T and atomic force microscopy methods indicated that AβMet35-sulfoxide impedes fibril formation [104–106]. Apparently, the presence of oxidized Met35 favors monomers and dimers over larger oligomers and enhances neurotoxicity [107]. Molecular dynamics simulations of Aβ suggest that Met35 oxidation decreases the β-strand content of the C-terminal hydrophobic domain of Aβ, specifically at the Aβ33-35 structural domain and that this configuration hinders Aβ polymerization [108].

Nitrosylation

Nitration at Tyr10 accelerates Aβ aggregation and has been detected in the amyloid plaques of both APP/PS1 mice and AD brains [109]. In a more recent study Aβtyr10 was found to significantly decrease Aβ aggregation and cytotoxicity [110].

The intriguing role of dimeric Aβ in AD pathology

In the 1990s the hypothetical cause of AD pathogenesis shifted from the insoluble fibrillar amyloid plaques to soluble oligomeric forms of Aβ. Substantial work has been dedicated to

understanding the physicochemical properties of Aβ aggregates ranging from dimers to large conglomerates [111–115]. In 1996, our group isolated detergent-free, water-soluble Aβ (n-40 and n-42) from normal and AD brains [112] in which the most prevalent and stable fraction was dimeric Aβ [113]. Amyloid-β dimers derived from AD amyloid plaques and vascular deposits were tested for toxicity in cultures of rat hippocampal neurons and glial cells [113]. Intriguingly, Aβ dimers elicited neuronal killing only in the presence of microglia. Amyloid-β dimers with PTM, including isoAsp1 and isoAsp7, cyclization of Glu3 to pyroglutamyl and oxidation of Met35, exhibit increased insolubility and stability. Amyloid-β1-42, with IsoAsp at positions 1 and 7, demonstrated the fastest rate of oligomerization, followed by Aβ3pE-42 and Aβ1-42. Amyloid-β1-40 showed a slower dimerization rate while Aβ1-28 did not dimerize [59]. Furthermore, tryptic digestion resistance progressively increases from Aβ1-40 monomer, Aβ1-42 monomer, Aβ3pE-42 monomer, Aβ1-42 (1,7 isoAsp) monomer, Aβ1-42 (1,7 isoAsp) dimer and Aβ17-42. Amyloid-β1-42 with oxidized Met35 to either Met sulfone or sulfoxide, was ~50% more resistant to digestion than non-oxidized Aβ1-42 [59]. These experiments suggest that the length of the Aβ peptides and PTM induce structural changes which impart unique physicochemical properties and functional effects.

Several dimeric and oligomeric Aβ models have been investigated in recent years (reviewed in reference [1]). Dimeric Aβ based on FASTA and BLAST SwissProt data using the PredictProtein and TOPITS algorithms yielded a Greek-key Aβ motif conformation in which four antiparallel β-strands generate a compact Aβ dimer with a hydrophobic core to shelter non-polar residues from the surrounding water [116]. In this model, the hydrophobic C-terminal domains of the Aβ dimer are thermodynamically shielded since they are partially buried along the dimer crevices, but can be extended to form the core of antiparallel β-sheets (see below). This model was further refined by molecular dynamics simulations [116]. Atomic force microscopy of purified dimers from amyloid plaques revealed the Aβ dimer as a compact globular hydrated structure ~35–38 Angstroms in diameter [113,116]. A series of studies suggests the importance of the stable soluble Aβ oligomers in AD cognitive dysfunction [116–119], conformational-dependent mechanisms of neurotoxicity [120], ability to induce tau hyperphosphorylation and neuronal degeneration [121] as well as stability in SDS solutions [34] with the latter property implicated in the generation of concentration-dependent dimers [122]. However, dimers have been purified in our laboratory in the absence of detergents [111]. Amyloid-β dimers isolated from the human brain impair synaptic plasticity and are detrimental to memory by inhibiting long-term potentiation, enhancing long-term depression and decreasing dendritic spine density in animal models [123]. Moreover, the degree of neurotoxicity is apparently dependent on the amount of Aβ dimers/trimers [124]. Recent experiments suggest that the binding of interstitial fluid Aβ oligomers to GM1 gangliosides produces destabilizing structural changes in membranes [125]. Synthetic dimeric Aβ inhibits mitochondrial cytochrome C-oxidase in the presence of copper [126]. Single-molecule atomic force microscopy experiments indicate that aggregation of Aβ is modulated by local environmental conditions and that Aβ42 dimerization is an extremely rapid process. In addition, the drastic structural differences between Aβ40 and Aβ42 may play a key role in dimerization propensity [127,128]. Amyloid-β dimers have also been proposed as the molecular unit in the polymerization of

amyloid fibrils. In this model based on cryo-EM, two opposing monomeric Aβ molecules comprising Aβ residues 25–41 generate a face-to-face antiparallel β-sheet by adopting an S-shape zipper-like hydrophobic core 'C-domain' while leaving the N-terminal regions, mostly composed of polar amino acids (residues 1–24), to make two opposing 'P-domains'. The subsequent stacking of these dimeric structures creates coiled two-stranded amyloid filaments [50]. It has been estimated that Aβ dimers are a million-fold more thermodynamically stable than disordered unstructured Aβ monomers [127].

The role of soluble oligomeric Aβ peptides

In recent years oligomers have been assumed to be the ultimate cause for synaptic dysfunction, neuroinflammation, neurovascular compromise and neuronal/glial degeneration, making them the target of intense research and immunotherapy interventions [20,22,129–134]. However, the notion of soluble oligomeric Aβ toxicity still deserves further scrutiny and comprehensive validation. One major problem is that the enormous diversity of the Aβ peptides influenced by PTM and peptide length also affects the size, biochemistry and biophysical properties of oligomers. Although Aβ dimers appear to be stable, larger Aβ oligomers have been isolated from mice and human brains using a variety of purification techniques. Oligomers might assume a very large number of conformational structures with a correspondingly huge diversity of epitopes. This complexity may explain why immunotherapies with antibodies assumed to be reacting with oligomers in the human brain have yielded poor results in clinical trials (reviewed in ref: [135]. There is no doubt that variable amounts of soluble monomeric and oligomeric Aβ exist in the human brain because metastable monomeric Aβ is continuously generated from APP by the action of secretases. There is also proof that, at least under controlled experimental conditions, oligomers are neurotoxic in cell culture and experimental animals [136–141]. However, the definition of Aβ oligomers is vague since different laboratories in academia and commercial settings produce their own unique varieties based on synthetic peptides and *in vitro* aggregation conditions. In most instances these oligomers, primarily built on unmodified full-length synthetic Aβ40 or Aβ42 amino acid sequences, have been assumed to be a faithful representation of what is present in the far more complex AD brain environment. In addition, Aβ oligomers have been extracted from animal or human brains using techniques that employ a diversity of mechanical homogenizing stresses. These extracted species may include artifacts from dispersed fibrillar Aβ which may not be present in the AD brain.

The complicated catalog of APP/Aβ-related peptides and AD amyloidosis

The profusion of amyloid plaques and their multiple morphological presentations suggests an underlying complexity in chemical compositions. A substantial mass of the amyloid plaque core is composed of a complex mixture of glycoproteins, glycolipids, lipids and proteins other than APP/Aβ [142,143]. Among the best characterized molecules are a variety of glycosaminoglycans, gangliosides, cholesterol, fatty acids, triglycerides, α1-antichymotrypsin and apolipoprotein E [144–152] and a large number of proteins identified by mass spectrometry [143,153]. Approximately 35% of the mass of AD amyloid cores is composed of non-Aβ molecules [32] enmeshed within an array of 10 nm fibrillar Aβ peptides. The biological function of the non-Aβ molecules in the context of plaque

pathology and dementia has never been investigated in detail. Based on the conventional notion that in AD amyloid plaques are mainly composed of unmodified Aβ1-40 and Aβ1-42 peptides, several therapeutic antibodies have been synthesized against short consecutive amino acid sequences of the intact N-terminal, C-terminal and middle domains of these peptides. Biochemical analyses of AD purified amyloid plaque cores have shown that the N-termini of Aβ are highly variable, probably resulting from aminopeptidase activity that is associated with degradation pathways of Aβ. In addition, BACE1, that normally cleaves APP to generate the amino terminus of Aβ1-40/42, can also cleave APP at residue Aβ11 to generate Aβ11-40/42 [154]. The proteolytic activity of the α-secretase on APP produces the "non-amyloidogenic" Aβ17-40/42, recognized as P3, which is abundant in diffuse amyloid plaques in cortical and cerebellar deposits [155–157]. These plaques have been deemed "non-fibrillar" but are known from thioflavine-S staining and EM studies to contain a low density of amyloid fibrils [158]. Due to its overall hydrophobic composition and insolubility P3 is very difficult to test in cell and animal models leaving the function of this peptide still unknown. However, because it is associated with diffuse plaques and may not elicit adjacent inflammatory reactions, P3 has been assumed to be an innocuous molecule. The potential ability of P3 to disrupt membrane lipids and form ionic channels implies this peptide may induce pathological changes in membrane permeability [159–161].

The Aβ C-termini are also variable [162]. It has been proposed that the γ-secretase primarily cleaves APP at residues Aβ48 and Aβ49, known as ε-sites, producing Aβ1-48 and Aβ1-49, and corresponding intracellular domains (AICD) 49–99 and 50–99 [163,164]. In addition, the γ-secretase can hydrolyze APP at residues Aβ46-47, the ζ-site [165], thus generating longer Aβ peptides [166–168]. The sequential hydrolysis of APP by γ-secretase in AD apparently generates a step-wise series of Aβ peptides terminating in residues 49, 48, 46, 45, 43, 42, 40, 39, 38 and 37 [163,164]. These Aβ forms have not been quantified in the AD brain. It is likely that the ratios of these Aβ peptides will vary from individual to individual. Interestingly, in the *PSEN1* EOAD mutation E280A (paisa) the Aβ C-termini are also heterogeneous with peptides ending at every position from residue 42 to residue 55 [169].

The traditional view that concerted processing of APP by the α, β and γ secretases produces Aβ amyloidogenic and non-amyloidogenic peptides is complicated by the recognition of alternative APP cleavage sites [170]. Some elongated Aβ-related peptides have been isolated and rigorously characterized by amino acid sequencing. Amyloid precursor protein hydrolysis at the δ-position Thr584 (APP_{695}) yields a product with an additional 12 amino acid residues extending from the N-terminus of the Aβ peptide [171]. More recently, two additional APP/Aβ peptides produced by an asparagine endopeptidase have been identified. Cleavage of APP_{695} at Asn373 creates an APP N-terminal neurotoxic peptide, and at Asn585 yields an APP C-terminal peptide, composed of residues 586–695 that serves as a preferred substrate for BACE1 [172]. It was further suggested that this latter peptide increases amyloid production, highlighting the potential importance of the δ-site in AD pathogenesis [172]. Another APP hydrolysis site, defined as the η-site, was discovered between residues 504–505 (APP_{695}). The η-peptide is further processed by the β- and α-secretases to create the Aη-β and Aη-α APP fragments. The latter peptide inhibited neuronal activity in the hippocampus by lowering long-term potentiation [173]. It has been suggested that cathepsin-L degrades the η-C-terminal fragment of APP [174]. In addition to

these APP-derived peptides, the APP C-terminal fragment containing the last 100 amino acids of APP (emulating β-secretase hydrolysis and absence of ɣ-secretase cleavage) induces neurodegeneration in transgenic mice [175,176]. Moreover, the AICD fragment can be further hydrolyzed to yield the Jcasp and the C31 peptides that have been found to induce apoptosis and have neurotoxic activity [177–180]. Lastly, APP-derived peptide carrying the N-terminal sequence of amino acid residues 18–286 was found to produce axonal pruning and neuronal death by interacting with the death receptor-6 (DR6) via the activation of caspases [181].

The evolutionary conservation of the APP and the redundancy generated by the amyloid precursor like-proteins (APLP1 and APLP2A) molecules is a testimony to its importance in modulating the function and fate of cells. The increased expression of APP is likely to generate an overproduction of specific peptides that may influence AD pathogenesis and development [182].

Implications of the AN-1792 active vaccination clinical trial

Neuropathological and biochemical examination of the brains of individuals actively vaccinated with aggregated synthetic Aβ1-42 + adjuvant (AN-1792) revealed neuritic and cored plaques were apparently disrupted while diffuse plaques and cerebrovascular amyloid were unaffected [183–187]. The cerebral cortex of vaccinated individuals showed a distinctive patchy distribution of neuritic and cored plaques with intercalation of adjacent plaque-poor and plaque-rich areas. In some individuals, the amyloid plaques left remnants suggestive of ‘collapsed plaques’ or ‘moth-eaten plaques’ that were reminiscent of the putative original plaque outline [183–187]. In some other instances, remnant structures exhibited a minuscule central deposit of amyloid surrounded by a clear area devoid of amyloid and a thin peripheral ‘halo’ of amyloid positive material [187]. ELISA analyses revealed the levels of water-soluble Aβ40 and Aβ42 were dramatically increased compared to a non-vaccinated AD population. In addition, vaccinated subjects had increased amounts of formic acid/guanidine hydrochloride-extractable Aβ40 coupled with a decrease in Aβ42 levels [188].

The above data suggest that, in some vaccinated individuals with high serum antibody titers, the anti-Aβ antibodies effectively crossed the blood-brain barrier (BBB) and reached their targets. These antibodies were capable of removing amyloid from plaque neuritic haloes and cores, probably from those mainly containing Aβ42. The interrupted pattern of plaque loss, however, indicates either variability in vascular antibody permeability or of their action on subtypes of amyloid deposits. Additionally, the patchy plaque elimination could be a consequence of treatment cessation since the trial was discontinued after some patients developed aseptic meningoencephalitis. Interestingly, Holmes et al. [189] reported that some cases exhibited an almost complete absence of histologically visible amyloid deposits. However, it is likely that some subjects never harbored amyloid deposits in the first place. For instance, case #14, described in reference [188], reported as having a complete absence of plaques had the lowest levels of Aβ formic acid extracted Aβ40 and Aβ42 and no soluble amyloid by immunoassays. However, this subject was Braak stage VI and likely an instance of a primary tauopathy such as progressive supranuclear palsy or corticobasal degeneration.

AN-1792 active vaccination was apparently far more effective at plaque disruption than passive immunizations with monoclonal antibodies. In the former case, multiple polyclonal antibodies recognized a large number of epitopes generated by different Aβ aggregated conformations. However, in most cases, the clearance of Aβ deposits was incomplete since diffuse plaques rich in Aβ17-42 (P3) and vascular-associated amyloid in cerebral cortex and leptomeningeal vessels, composed primarily of Aβ40, were unaffected. Despite the apparent effectiveness of AN-1792 in disrupting at least some amyloid plaques, this therapy notably failed to halt cognitive impairment progression [189].

Peripheral Aβ

Amyloid precursor protein is expressed in most human cells suggesting peptides derived from this molecule, including Aβ, exist in most tissues and compartments of the body. In addition to the uncertainty over the temporal pace of Aβ deposition and the sequential location of brain affected sites, the role of Aβ in circulating plasma and CSF in the development of AD remains enigmatic. Circulating Aβ is predominately bound to albumin and other plasma molecules [190–192]. Amyloid-β has been detected in peripheral tissues [193]. For example, in skeletal muscle the levels of Aβ42 and total Aβ are significantly elevated in AD when compared to non-demented controls. Like the brain, skeletal muscle, which represents about one-third of the body mass, also generates a diverse array of Aβ peptides [194]. Furthermore, the aortas of elderly individuals with severe atherosclerotic deposits contain twice the amount of total Aβ40 and Aβ42 than subjects with minimal atherosclerotic vascular disease [195]. Another important source of peripheral Aβ are the platelets. Quiescent platelets contain more Aβ40 than activated de-granulated ones [193]. The administration of anti-Aβ antibody infusions are likely to have some effect on the levels of circulating Aβ generated in peripheral tissues. Hence, any therapeutic interventions against AD amyloidosis relying only on the levels of circulating Aβ levels to measure their efficacy may lead to erroneous interpretations. Whether or not circulating Aβ contributes to the brain pool of these molecules remains to be answered with certainty. The physiologic and health implications of perturbing peripheral Aβ pools on a chronic basis are unknown.

Future biomarker discovery and immunotherapy tactics

While many studies have confirmed the role of Aβ in AD pathology, there is considerable confusion as to which of its myriad forms will provide effective diagnostic markers and therapeutic targets. Numerous lines of evidence have implicated various Aβ species including soluble, oligomeric, globular or annular aggregates [196–203] as critical players in synaptic demise and early memory loss of AD. Likewise, there is no consensus regarding the form(s) of covalently modified Aβ most intimately involved in neurological decline. There is also considerable uncertainty over where Aβ accumulation first occurs in the brain and whether the deposited molecules are generated within the brain exclusively or augmented by peripheral pools. Under normal circumstances Aβ is proteolytically degraded in brain or cleared by the liver and kidneys [204–206], but very little is known about the catabolism of the PTM Aβ peptides. Adding to these complexities, a variety of homogeneous or heterogeneous aggregated Aβ species could be stochastically generated in brain tissue. In some regions of the AD brain up to 12 copies of the APP gene have been found in some

neurons. Expression of all or some of these APP genes may participate the pathogenesis of AD [207,208]. Different Aβ peptide species may play distinct roles that are dictated by their specific molecular conformations.

Identification of Aβ related antibodies that selectively recognize conformational epitopes in different AD patients is an ideal approach for the development of biomarkers and therapeutic agents. Antibodies against Aβ oligomers have been utilized to confirm the existence and role of oligomeric Aβ species [118,197,198,209–211]. The most useful Aβ antibodies for biomarker discovery might be those targeting specific epitopes on molecules known to be widely distributed in AD subjects.

Novel methods have achieved this goal by combining the imaging capabilities of atomic force microscopy with phage display antibody technology which enables the identification of specific protein variants and isolation of reagents that selectively bind the target protein [212]. These technologies permit the generation of antibody based (nanobody) reagents that preferentially differentiate toxic-disease associated variants of key neuronal proteins including Aβ, tau, TDP43 and α-synuclein [212–221]. In the case of Aβ, nanobodies revealed three conformationally distinct oligomeric variants that differentiate postmortem AD brain specimens from healthy or Parkinson's disease cases [220,222–224]. These observations indicate that detection of disease related protein variants may be a powerful blood or CSF based biomarker tool for AD and related neurodegenerative diseases. Since Aβ is such a complex protein and AD is a heterogeneous disease, detection of specific Aβ variants and other related deviant proteins have great promise as individualized biomarkers for AD and great potential for precision-personalized medicine.

Conclusions

At the center of the AD-amyloid conundrum is the unresolved observation that in the absence of genetic mutations Aβ peptides spontaneously aggregate into amyloid plaques and the walls of the cerebral vasculature. We contend this apparently spontaneous change is enhanced by alterations gene expression and PTM of the Aβ peptide structures which increases their stability and promotes their preferential propagation throughout the brain.

It is unclear whether the widely accepted assumption that unmodified, full length Aβ40/Aβ42/Aβ43 and their soluble/oligomeric/fibrillary forms are the main culprits responsible for the pathology and clinical manifestations of late-onset AD. Experimental investigations reveal the Aβ molecules harbored by AD subjects are structurally diverse with different conformations and biological properties. However, to date most passive Aβ immunotherapies, with the exception of aducanumab, have targeted relatively short linear Aβ1-42 amino acid sequences rather than specifically folded tertiary structures.

Mounting evidence suggests that pathologic prions derived from normal proteins underlie several neurologic diseases including AD. Prion strains exhibit unique biochemical properties imparted by specific toxic molecular conformations and these strain-specific pathologic structures are faithfully replicated [225]. Conformational alterations induced by PTM of Aβ to yield unique amyloid strains may partially account for the clinical and

pathological heterogeneity of LOAD [225]. Analogous to situations in which transmissible prions cross species barriers, the modified Aβ molecules of AD subjects would induce to adopt and propagate the specific toxic conformation of spontaneously emerging pathologic seeds. Self-transmissible Aβ strains capable of inducing distinct pathologic manifestations have been isolated from AD subjects [225].

To date, Aβ physical diversity and functional significance of 3D conformations to dementia emergence and neurotoxicity have been almost ignored. In addition to these differing biophysical features among Aβ species, quantitative differences in the proclivity to accumulate may also contribute to their pathological oligomerization and deposition in the aging brain. It can be assumed that some of these Aβ-related molecules have positive adaptive functions while others may be detrimental to brain homeostasis. Several lines of circumstantial and experimental evidence have suggested that under damaging conditions such as brain trauma, microbial invasion, a leaky blood-brain barrier and hypertensive crisis, sustained overproduction of some Aβ peptides may have a rescue function. This assumption is supported by the molecular conservation of the Aβ amino acid sequence along mammalian evolution that suggests important adaptive values for these peptides. It is still unclear which Aβ alternatives, including PTM peptides, are involved in the onset and progression of AD and thus might represent the best therapeutic targets, or, alternatively, which may have a salvage function.

We propose that amplified production of some Aβ species, probably complicated by reduced proteolytic degradation occurring during aging, creates a diverse spectrum of molecules which ultimately disrupt brain homeostasis and contribute to AD neurodegeneration. We postulate that the generation of some specific 3D-peptide conformations of Aβ impart a unique array of biophysical properties with deleterious as well as protective effects. Proteolytic processing of the highly evolutionarily-conserved multifunctional APP molecule is capable of creating over a dozen of proteolytically-derived peptides which are involved in a large number of brain functions, some of them with deleterious properties. The APP dynamics must be finely tuned through transcription and translation and closely regulated in terms of proteolytic processing and degradation. In addition to Aβ, the excessive production of multiple neurotoxic peptides derived from the proteolysis of APP may play important roles in the development of late-onset AD. Some of these APP peptides may be involved in the initial stages of AD and could have profound effects in subsequent neurodegeneration.

One factor confounding the interpretation of previous clinical trials is the observation that a large fraction of elderly dementia cases, even those with clinical manifestations of AD do not harbor conventionally defined AD neuropathology based on densities and distributions of plaques and tangles [226]. The A/T/N classification scheme of Jack *et al.* [227] proposes to integrate additional markers of neurodegeneration into a nosological partition of AD and other dementias, helping to define clinical subgroups. Coupled with imaging methods capable of revealing amyloid and tangle deposits in living subjects and correlated with clinical signs and symptoms, this more nuanced view of dementia may aid in the design and interpretation of future clinical trials.

Advances in imaging techniques, genetics and neurochemistry will further enable investigators to classify demented subjects on the basis of amyloid or tau deposition patterns with unprecedented precision. Sophisticated, minimally-invasive biopsy methods [228], coupled with innovative analytical techniques would help clarify the effects of Aβ molecular diversity on pathogenesis and aid in the identification of additional pathologies including tau, α-synuclein and TDP-43. Longitudinal studies combining imaging, molecular fingerprinting and cognitive function exams may reveal if the kinetics assumed for the amyloid cascade hypothesis holds for the majority or only a limited number of AD demented subjects. Clarifying which of the structurally altered Aβ peptides are responsible for neurotoxicity will help in the design of specific therapeutic interventions. Reagents that selectively recognize and target different Aβ conformational variants will be powerful tools to assist in the individual diagnosis and personalized treatment of AD patients. Detailed examinations of the non-demented oldest-old subjects retaining cognitive function while harboring the neuropathologic lesions of AD may help reveal which amyloid species are inimical to neuronal and vascular function and which may be comparatively less toxic or non-toxic.

Acknowledgments

This study was supported by: The National Institute on Aging grants R01 AG019795 (AER), Midwestern University, Glendale, AZ (TAK), The Life Extension Foundation, Inc. and the Elizabeth and Thomas Plott Chair in Gerontology of the UCLA Longevity Center (SGC), and the Arizona Alzheimer's Disease Consortium and from the Department of Defense (W81XWH-14-1-0467) (MRS). Detailed Abeta studies were made possible by the Brain and Body Donation Program at Banner Sun Health Research Institute, which has been supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson's Disease Consortium) and the Michael J. Fox Foundation for Parkinson's Research. The funders had no role in study design, data collection, analysis or interpretation of data, decision to publish or preparation of the manuscript.

Sabbagh MS is a consultant for: Axovant, Biogen, Grifols, Humana, Lilly pharmaceuticals, Sanofi and vTv Therapeutics. He receives research grant support from: Astra Seneca, Avid Pharmaceuticals, Axovant, Genentech Inc., Lilly Pharmaceuticals, Merck and Co., Pfizer, Roche Diagnostics Corp., vTv Therapeutics and Piramal Imaging. He is a stock shareholder of Brain Health, Muses Labs., and Versanum.

Beach TG is an advisory board member for Genentech, consultant for Avid Radiopharmaceuticals and GE Healthcare, has research contracts with Avid Radiopharmaceuticals and Navidea Biopharmaceuticals, and receives grant support from the National Institutes of Health, the Michael J. Fox Foundation for Parkinson's Research and the State of Arizona.

Reference List

1. Masters CL, Selkoe DJ. Biochemistry of amyloid beta-protein and amyloid deposits in Alzheimer disease. Cold Spring Harb Perspect Med. 2012; 2:a006262. [PubMed: 22675658]
2. Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. Neuron. 1992; 8:159–168. [PubMed: 1530909]
3. Shivers BD, Hilbich C, Multhaup G, Salbaum M, Beyreuther K, Seeburg PH. Alzheimer's disease amyloidogenic glycoprotein: expression pattern in rat brain suggests a role in cell contact. EMBO J. 1988; 7:1365–1370. [PubMed: 2900758]
4. Roychaudhuri R, Zheng X, Lomakin A, Maiti P, Condron MM, Benedek GB, Bitan G, Bowers MT, Teplow DB. Role of Species-Specific Primary Structure Differences in Abeta42 Assembly and Neurotoxicity. ACS Chem Neurosci. 2015; 6:1941–1955. [PubMed: 26421877]

5. Fung J, Frost D, Chakrabartty A, McLaurin J. Interaction of human and mouse Abeta peptides. J Neurochem. 2004; 91:1398–1403. [PubMed: 15584916]
6. Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K. Human and rodent sequence analogs of Alzheimer's amyloid beta A4 share similar properties and can be solubilized in buffers of pH 7.4. Eur J Biochem. 1991; 201:61–69. [PubMed: 1915378]
7. Herrup K. The case for rejecting the amyloid cascade hypothesis. Nat Neurosci. 2015; 18:794–799. [PubMed: 26007212]
8. Lee HG, Zhu X, Nunomura A, Perry G, Smith MA. Amyloid beta: the alternate hypothesis. Curr Alzheimer Res. 2006; 3:75–80. [PubMed: 16472207]
9. Mullane K, Williams M. Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol. 2013; 85:289–305. [PubMed: 23178653]
10. Chetelat G. Alzheimer disease: Abeta-independent processes-rethinking preclinical AD. Nat Rev Neurol. 2013; 9:123–124. [PubMed: 23399647]
11. Jack CR Jr, Knopman DS, Chetelat G, Dickson D, Fagan AM, Frisoni GB, Jagust W, Mormino EC, Petersen RC, Sperling RA, van Der Flier WM, Villemagne VL, Visser PJ, Vos SJ. Suspected non-Alzheimer disease pathophysiology -concept and controversy. Nat Rev Neurol. 2016; 12:117–124. [PubMed: 26782335]
12. Josephs KA, Whitwell JL, Ahmed Z, Shiung MM, Weigand SD, Knopman DS, Boeve BF, Parisi JE, Petersen RC, Dickson DW, Jack CR Jr. Beta-amyloid burden is not associated with rates of brain atrophy. Ann Neurol. 2008; 63:204–212. [PubMed: 17894374]
13. Prohovnik I, Perl DP, Davis KL, Libow L, Lesser G, Haroutunian V. Dissociation of neuropathology from severity of dementia in late-onset Alzheimer disease. Neurology. 2006; 66:49–55. [PubMed: 16401845]
14. Villemagne VL, Pike KE, Chetelat G, Ellis KA, Mulligan RS, Bourgeat P, Ackermann U, Jones G, Szoeke C, Salvado O, Martins R, O'Keefe G, Mathis CA, Klunk WE, Ames D, Masters CL, Rowe CC. Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. Ann Neurol. 2011; 69:181–192. [PubMed: 21280088]
15. Wolk DA. Amyloid imaging in atypical presentations of Alzheimer's disease. Curr Neurol Neurosci Rep. 2013; 13:412. [PubMed: 24136459]
16. Boyle PA, Wilson RS, Yu L, Barr AM, Honer WG, Schneider JA, Bennett DA. Much of late life cognitive decline is not due to common neurodegenerative pathologies. Ann Neurol. 2013; 74:478–489. [PubMed: 23798485]
17. Nelson PT, Abner EL, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Davis DG, Poduska JW, Patel E, Mendiondo MS, Markesbery WR. Modeling the association between 43 different clinical and pathological variables and the severity of cognitive impairment in a large autopsy cohort of elderly persons. Brain Pathol. 2010; 20:66–79. [PubMed: 19021630]
18. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ, Davies P, Del Tredici K, Duyckaerts C, Frosch MP, Haroutunian V, Hof PR, Hulette CM, Hyman BT, Iwatsubo T, Jellinger KA, Jicha GA, Kovari E, Kukull WA, Leverenz JB, Love S, Mackenzie IR, Mann DM, Masliah E, McKee AC, Montine TJ, Morris JC, Schneider JA, Sonnen JA, Thal DR, Trojanowski JQ, Troncoso JC, Wisniewski T, Woltjer RL, Beach TG. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol. 2012; 71:362–381. [PubMed: 22487856]
19. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ, Davies P, Del TK, Duyckaerts C, Frosch MP, Haroutunian V, Hof PR, Hulette CM, Hyman BT, Iwatsubo T, Jellinger KA, Jicha GA, Kovari E, Kukull WA, Leverenz JB, Love S, Mackenzie IR, Mann DM, Masliah E, McKee AC, Montine TJ, Morris JC, Schneider JA, Sonnen JA, Thal DR, Trojanowski JQ, Troncoso JC, Wisniewski T, Woltjer RL, Beach TG. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol. 2012; 71:362–381. [PubMed: 22487856]
20. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016

21. Tu S, Okamoto S, Lipton SA, Xu H. Oligomeric Abeta-induced synaptic dysfunction in Alzheimer's disease. Mol Neurodegener. 2014; 9:48. [PubMed: 25394486]
22. Watson D, Castano E, Kokjohn TA, Kuo YM, Lyubchenko Y, Pinsky D, Connolly ES Jr, Esh C, Luehrs DC, Stine WB, Rowse LM, Emmerling MR, Roher AE. Physicochemical characteristics of soluble oligomeric Abeta and their pathologic role in Alzheimer's disease. Neurol Res. 2005; 27:869–881. [PubMed: 16354549]
23. Williams TL, Serpell LC. Membrane and surface interactions of Alzheimer's Abeta peptide–insights into the mechanism of cytotoxicity. FEBS J. 2011; 278:3905–3917. [PubMed: 21722314]
24. LaFerla FM, Green KN, Oddo S. Intracellular amyloid-beta in Alzheimer's disease. Nat Rev Neurosci. 2007; 8:499–509. [PubMed: 17551515]
25. Carnevale D, Mascio G, D'Andrea I, Fardella V, Bell RD, Branchi I, Pallante F, Zlokovic B, Yan SS, Lembo G. Hypertension induces brain beta-amyloid accumulation, cognitive impairment, and memory deterioration through activation of receptor for advanced glycation end products in brain vasculature. Hypertension. 2012; 60:188–197. [PubMed: 22615109]
26. Deane R, Du YS, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Stern A, Schmidt AM, Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D, Zlokovic B. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nat Med. 2003; 9:907–913. [PubMed: 12808450]
27. Eisele YS, Fritschi SK, Hamaguchi T, Obermuller U, Fuger P, Skodras A, Schafer C, Odenthal J, Heikenwalder M, Staufenbiel M, Jucker M. Multiple factors contribute to the peripheral induction of cerebral beta-amyloidosis. J Neurosci. 2014; 34:10264–10273. [PubMed: 25080588]
28. Mackic JB, Bading J, Ghiso J, Walker L, Wisniewski T, Frangione B, Zlokovic BV. Circulating amyloid-beta peptide crosses the blood-brain barrier in aged monkeys and contributes to Alzheimer's disease lesions. Vascul Pharmacol. 2002; 38:303–313. [PubMed: 12529925]
29. Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature. 2013; 501:45–51. [PubMed: 24005412]
30. Prusiner SB. Cell biology. A unifying role for prions in neurodegenerative diseases. Science. 2012; 336:1511–1513. [PubMed: 22723400]
31. Prusiner SB. Biology and genetics of prions causing neurodegeneration. Annu Rev Genet. 2013; 47:601–623. [PubMed: 24274755]
32. Roher AE, Lowenson JD, Clarke S, Wolkow C, Wang R, Cotter RJ, Reardon IM, Zurcher-Neely HA, Heinrikson RL, Ball MJ. Structural alterations in the peptide backbone of beta-amyloid core protein may account for its deposition and stability in Alzheimer's disease. J Biol Chem. 1993; 268:3072–3083. [PubMed: 8428986]
33. Kummer MP, Heneka MT. Truncated and modified amyloid-beta species. Alzheimers Res Ther. 2014; 6:28. [PubMed: 25031638]
34. McDonald JM, Cairns NJ, Taylor-Reinwald L, Holtzman D, Walsh DM. The levels of water-soluble and triton-soluble Abeta are increased in Alzheimer's disease brain. Brain Res. 2012; 1450:138–147. [PubMed: 22440675]
35. Geiger T, Clarke S. Deamidation, isomerization, and racemization at asparaginyl and aspartyl residues in peptides. Succinimide-linked reactions that contribute to protein degradation. J Biol Chem. 1987; 262:785–794. [PubMed: 3805008]
36. Radkiewicz JL, Zipse H, Clarke S, Houk KN. Accelerated racemization of aspartic acid and asparagine residues via succinimide intermediates: an ab initio theoretical exploration of mechanism. 1996; 118:9148–9155.
37. Tambo K, Yamaguchi T, Kobayashi K, Terauchi E, Ichi I, Kojo S. Racemization of the aspartic acid residue of amyloid-beta peptide by a radical reaction. Biosci Biotechnol Biochem. 2013; 77:416–418. [PubMed: 23391937]
38. Lowenson JD, Clarke S. Structural elements affecting the recognition of L-isoaspartyl residues by the L-isoaspartyl/D-aspartyl protein methyltransferase. Implications for the repair hypothesis. J Biol Chem. 1991; 266:19396–19406. [PubMed: 1833402]

39. Fukuda H, Shimizu T, Nakajima M, Mori H, Shirasawa T. Synthesis, aggregation, and neurotoxicity of the Alzheimer's Abeta1-42 amyloid peptide and its isoaspartyl isomers. Bioorg Med Chem Lett. 1999; 9:953–956. [PubMed: 10230618]
40. Shimizu T, Watanabe A, Ogawara M, Mori H, Shirasawa T. Isoaspartate formation and neurodegeneration in Alzheimer's disease. Arch Biochem Biophys. 2000; 381:225–234. [PubMed: 11032409]
41. Shimizu T, Matsuoka Y, Shirasawa T. Biological significance of isoaspartate and its repair system. Biol Pharm Bull. 2005; 28:1590–1596. [PubMed: 16141521]
42. Fossati S, Todd K, Sotolongo K, Ghiso J, Rostagno A. Differential contribution of isoaspartate post-translational modifications to the fibrillization and toxic properties of amyloid beta and the Asn23 Iowa mutation. Biochem J. 2013; 456:347–360. [PubMed: 24028142]
43. Shin Y, Cho HS, Fukumoto H, Shimizu T, Shirasawa T, Greenberg SM, Rebeck GW. Abeta species, including IsoAsp23 Abeta, in Iowa-type familial cerebral amyloid angiopathy. Acta Neuropathol. 2003; 105:252–258. [PubMed: 12557012]
44. Tomidokoro Y, Rostagno A, Neubert TA, Lu Y, Rebeck GW, Frangione B, Greenberg SM, Ghiso J. Iowa variant of familial Alzheimer's disease: accumulation of posttranslationally modified AbetaD23N in parenchymal and cerebrovascular amyloid deposits. Am J Pathol. 2010; 176:1841–1854. [PubMed: 20228223]
45. Alies B, Eury H, Bijani C, Rechignat L, Faller P, Hureau C. pH-Dependent Cu(II) coordination to amyloid-beta peptide: impact of sequence alterations, including the H6R and D7N familial mutations. Inorg Chem. 2011; 50:11192–11201. [PubMed: 21980910]
46. Hori Y, Hashimoto T, Wakutani Y, Urakami K, Nakashima K, Condron MM, Tsubuki S, Saido TC, Teplow DB, Iwatsubo T. The Tottori (D7N) and English (H6R) familial Alzheimer disease mutations accelerate Abeta fibril formation without increasing protofibril formation. J Biol Chem. 2007; 282:4916–4923. [PubMed: 17170111]
47. Ono K, Condron MM, Teplow DB. Effects of the English (H6R) and Tottori (D7N) familial Alzheimer disease mutations on amyloid beta-protein assembly and toxicity. J Biol Chem. 2010; 285:23186–23197. [PubMed: 20452980]
48. Viet MH, Nguyen PH, Ngo ST, Li MS, Derreumaux P. Effect of the Tottori familial disease mutation (D7N) on the monomers and dimers of Abeta40 and Abeta42. ACS Chem Neurosci. 2013; 4:1446–1457. [PubMed: 24041307]
49. Roher AE, Lowenson JD, Clarke S, Woods AS, Cotter RJ, Gowing E, Ball MJ. beta-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. Proc Natl Acad Sci U S A. 1993; 90:10836–10840. [PubMed: 8248178]
50. Schmidt M, Rohou A, Lasker K, Yadav JK, Schiene-Fischer C, Fandrich M, Grigorieff N. Peptide dimer structure in an Abeta(1-42) fibril visualized with cryo-EM. Proc Natl Acad Sci U S A. 2015; 112:11858–11863. [PubMed: 26351699]
51. Jung G, Ryu J, Heo J, Lee SJ, Cho JY, Hong S. Protein L-isoaspartyl O-methyltransferase inhibits amyloid beta fibrillogenesis in vitro. Pharmazie. 2011; 66:529–534. [PubMed: 21812329]
52. Yang H, Fung EY, Zubarev AR, Zubarev RA. Toward proteome-scale identification and quantification of isoaspartyl residues in biological samples. J Proteome Res. 2009; 8:4615–4621. [PubMed: 19663459]
53. Inoue K, Hosaka D, Mochizuki N, Akatsu H, Tsutsumiuchi K, Hashizume Y, Matsukawa N, Yamamoto T, Toyo'oka T. Simultaneous determination of post-translational racemization and isomerization of N-terminal amyloid-beta in Alzheimer's brain tissues by covalent chiral derivatized ultraperformance liquid chromatography tandem mass spectrometry. Anal Chem. 2014; 86:797–804. [PubMed: 24283798]
54. Fabian H, Szendrei GI, Mantsch HH, Greenberg BD, Otvos L Jr. Synthetic post-translationally modified human A beta peptide exhibits a markedly increased tendency to form beta-pleated sheets in vitro. Eur J Biochem. 1994; 221:959–964. [PubMed: 8181478]
55. Orpiszewski J, Benson MD. Induction of beta-sheet structure in amyloidogenic peptides by neutralization of aspartate: a model for amyloid nucleation. J Mol Biol. 1999; 289:413–428. [PubMed: 10366514]

56. Szendrei GI, Fabian H, Mantsch HH, Lovas S, Nyeki O, Schon I, Otvos L Jr. Aspartate-bond isomerization affects the major conformations of synthetic peptides. Eur J Biochem. 1994; 226:917–924. [PubMed: 7813483]
57. Szendrei GI, Prammer KV, Vasko M, Lee VM, Otvos L Jr. The effects of aspartic acid-bond isomerization on in vitro properties of the amyloid beta-peptide as modeled with N-terminal decapeptide fragments. Int J Pept Protein Res. 1996; 47:289–296. [PubMed: 8738655]
58. Bohme L, Hoffmann T, Manhart S, Wolf R, Demuth HU. Isoaspartate-containing amyloid precursor protein-derived peptides alter efficacy and specificity of potential beta-secretases. Biol Chem. 2008; 389:1055–1066. [PubMed: 18979630]
59. Kuo YM, Webster S, Emmerling MR, De Lima N, Roher AE. Irreversible dimerization/tetramerization and post-translational modifications inhibit proteolytic degradation of A beta peptides of Alzheimer's disease. Biochim Biophys Acta. 1998; 1406:291–298. [PubMed: 9630681]
60. Lee JM, Petrucelli L, Fisher G, Ramdath S, Castillo J, Di Fiore MM, D'Aniello A. Evidence for D-aspartyl-beta-amyloid secretase activity in human brain. J Neuropathol Exp Neurol. 2002; 61:125–131. [PubMed: 11855381]
61. Iwatsubo T, Saido TC, Mann DM, Lee VM, Trojanowski JQ. Full-length amyloid-beta (1-42(43)) and amino-terminally modified and truncated amyloid-beta 42(43) deposit in diffuse plaques. Am J Pathol. 1996; 149:1823–1830. [PubMed: 8952519]
62. Mori H, Takio K, Ogawara M, Selkoe DJ. Mass spectrometry of purified amyloid beta protein in Alzheimer's disease. J Biol Chem. 1992; 267:17082–17086. [PubMed: 1512246]
63. Mandler M, Rockenstein E, Ubhi K, Hansen L, Adame A, Michael S, Galasko D, Santic R, Mattner F, Masliah E. Detection of peri-synaptic amyloid-beta pyroglutamate aggregates in early stages of Alzheimer's disease and in AbetaPP transgenic mice using a novel monoclonal antibody. J Alzheimers Dis. 2012; 28:783–794. [PubMed: 22064070]
64. De Kimpe L, van Haastert ES, Kaminari A, Zwart R, Rutjes H, Hoozemans JJ, Scheper W. Intracellular accumulation of aggregated pyroglutamate amyloid beta: convergence of aging and Abeta pathology at the lysosome. Age (Dordr). 2013; 35:673–687. [PubMed: 22477259]
65. Kuo YM, Emmerling MR, Woods AS, Cotter RJ, Roher AE. Isolation, chemical characterization, and quantitation of A beta 3-pyroglutamyl peptide from neuritic plaques and vascular amyloid deposits. Biochem Biophys Res Commun. 1997; 237:188–191. [PubMed: 9266855]
66. Perez-Garmendia R, Gevorkian G. Pyroglutamate-Modified Amyloid Beta Peptides: Emerging Targets for Alzheimer s Disease Immunotherapy. Curr Neuropharmacol. 2013; 11:491–498. [PubMed: 24403873]
67. D'Arrigo C, Tabaton M, Perico A. N-terminal truncated pyroglutamyl beta amyloid peptide Abetapy3-42 shows a faster aggregation kinetics than the full-length Abeta1-42. Biopolymers. 2009; 91:861–873. [PubMed: 19562755]
68. Gunn AP, Masters CL, Cherny RA. Pyroglutamate-Abeta: role in the natural history of Alzheimer's disease. Int J Biochem Cell Biol. 2010; 42:1915–1918. [PubMed: 20833262]
69. Harigaya Y, Saido TC, Eckman CB, Prada CM, Shoji M, Younkin SG. Amyloid beta protein starting pyroglutamate at position 3 is a major component of the amyloid deposits in the Alzheimer's disease brain. Biochem Biophys Res Commun. 2000; 276:422–427. [PubMed: 11027491]
70. He W, Barrow CJ. The A beta 3-pyroglutamyl and 11-pyroglutamyl peptides found in senile plaque have greater beta-sheet forming and aggregation propensities in vitro than full-length A beta. Biochemistry. 1999; 38:10871–10877. [PubMed: 10451383]
71. Hosoda R, Saido TC, Otvos L Jr, Arai T, Mann DM, Lee VM, Trojanowski JQ, Iwatsubo T. Quantification of modified amyloid beta peptides in Alzheimer disease and Down syndrome brains. J Neuropathol Exp Neurol. 1998; 57:1089–1095. [PubMed: 9825946]
72. Jawhar S, Wirths O, Bayer TA. Pyroglutamate amyloid-beta (Abeta): a hatchet man in Alzheimer disease. J Biol Chem. 2011; 286:38825–38832. [PubMed: 21965666]
73. Jawhar S, Trawicka A, Jenneckens C, Bayer TA, Wirths O. Motor deficits, neuron loss, and reduced anxiety coinciding with axonal degeneration and intraneuronal Abeta aggregation in the 5XFAD mouse model of Alzheimer's disease. Neurobiol Aging. 2012; 33:196–40.

74. Miravalle L, Calero M, Takao M, Roher AE, Ghetti B, Vidal R. Amino-terminally truncated Abeta peptide species are the main component of cotton wool plaques. Biochemistry. 2005; 44:10810–10821. [PubMed: 16086583]
75. Naslund J, Karlstrom AR, Tjernberg LO, Schierhorn A, Terenius L, Nordstedt C. High-resolution separation of amyloid beta-peptides: structural variants present in Alzheimer's disease amyloid. J Neurochem. 1996; 67:294–301. [PubMed: 8667005]
76. Nussbaum JM, Schilling S, Cynis H, Silva A, Swanson E, Wangsanut T, Tayler K, Wiltgen B, Hatami A, Ronicke R, Reymann K, Hutter-Paier B, Alexandru A, Jagla W, Graubner S, Glabe CG, Demuth HU, Bloom GS. Prion-like behaviour and tau-dependent cytotoxicity of pyroglutamylated amyloid-beta. Nature. 2012; 485:651–655. [PubMed: 22660329]
77. Pike CJ, Overman MJ, Cotman CW. Amino-terminal deletions enhance aggregation of beta-amyloid peptides in vitro. J Biol Chem. 1995; 270:23895–23898. [PubMed: 7592576]
78. Portelius E, Bogdanovic N, Gustavsson MK, Volkmann I, Brinkmalm G, Zetterberg H, Winblad B, Blennow K. Mass spectrometric characterization of brain amyloid beta isoform signatures in familial and sporadic Alzheimer's disease. Acta Neuropathol. 2010; 120:185–193. [PubMed: 20419305]
79. Russo C, Saido TC, DeBusk LM, Tabaton M, Gambetti P, Teller JK. Heterogeneity of water-soluble amyloid beta-peptide in Alzheimer's disease and Down's syndrome brains. FEBS Lett. 1997; 409:411–416. [PubMed: 9224700]
80. Russo C, Violani E, Salis S, Venezia V, Dolcini V, Damonte G, Benatti U, D'Arrigo C, Patrone E, Carlo P, Schettini G. Pyroglutamate-modified amyloid beta-peptides–AbetaN3(pE)–strongly affect cultured neuron and astrocyte survival. J Neurochem. 2002; 82:1480–1489. [PubMed: 12354296]
81. Saido TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S. Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. Neuron. 1995; 14:457–466. [PubMed: 7857653]
82. Schilling S, Lauber T, Schaupp M, Manhart S, Scheel E, Bohm G, Demuth HU. On the seeding and oligomerization of pGlu-amyloid peptides (in vitro). Biochemistry. 2006; 45:12393–12399. [PubMed: 17029395]
83. Sergeant N, Bombois S, Ghestem A, Drobecq H, Kostanjevecki V, Missiaen C, Wattez A, David JP, Vanmechelen E, Sergheraert C, Delacourte A. Truncated beta-amyloid peptide species in pre-clinical Alzheimer's disease as new targets for the vaccination approach. J Neurochem. 2003; 85:1581–1591. [PubMed: 12787077]
84. Sun N, Hartmann R, Lecher J, Stoldt M, Funke SA, Gremer L, Ludwig HH, Demuth HU, Kleinschmidt M, Willbold D. Structural analysis of the pyroglutamate-modified isoform of the Alzheimer's disease-related amyloid-beta using NMR spectroscopy. J Pept Sci. 2012; 18:691–695. [PubMed: 23001756]
85. Tekirian TL, Saido TC, Markesbery WR, Russell MJ, Wekstein DR, Patel E, Geddes JW. N-terminal heterogeneity of parenchymal and cerebrovascular Abeta deposits. J Neuropathol Exp Neurol. 1998; 57:76–94. [PubMed: 9600199]
86. Wirths O, Erck C, Martens H, Harmeier A, Geumann C, Jawhar S, Kumar S, Multhaup G, Walter J, Ingelsson M, Degerman-Gunnarsson M, Kalimo H, Huitinga I, Lannfelt L, Bayer TA. Identification of low molecular weight pyroglutamate A{beta} oligomers in Alzheimer disease: a novel tool for therapy and diagnosis. J Biol Chem. 2010; 285:41517–41524. [PubMed: 20971852]
87. Youssef I, Florent-Bechard S, Malaplate-Armand C, Koziel V, Bihain B, Olivier JL, Leininger-Muller B, Kriem B, Oster T, Pillot T. N-truncated amyloid-beta oligomers induce learning impairment and neuronal apoptosis. Neurobiol Aging. 2008; 29:1319–1333. [PubMed: 17459527]
88. DeMattos RB, Lu J, Tang Y, Racke MM, DeLong CA, Tzaferis JA, Hole JT, Forster BM, McDonnell PC, Liu F, Kinley RD, Jordan WH, Hutton ML. A plaque-specific antibody clears existing beta-amyloid plaques in Alzheimer's disease mice. Neuron. 2012; 76:908–920. [PubMed: 23217740]
89. Frost JL, Liu B, Kleinschmidt M, Schilling S, Demuth HU, Lemere CA. Passive immunization against pyroglutamate-3 amyloid-beta reduces plaque burden in Alzheimer-like transgenic mice: a pilot study. Neurodegener Dis. 2012; 10:265–270. [PubMed: 22343072]

90. Venkataramani V, Wirths O, Budka H, Hartig W, Kovacs GG, Bayer TA. Antibody 9D5 recognizes oligomeric pyroglutamate amyloid-beta in a fraction of amyloid-beta deposits in Alzheimer's disease without cross-reactivity with other protein aggregates. J Alzheimers Dis. 2012; 29:361–371. [PubMed: 22232007]
91. Schlenzig D, Ronicke R, Cynis H, Ludwig HH, Scheel E, Reymann K, Saido T, Hause G, Schilling S, Demuth HU. N-Terminal pyroglutamate formation of Abeta38 and Abeta40 enforces oligomer formation and potency to disrupt hippocampal long-term potentiation. J Neurochem. 2012; 121:774–784. [PubMed: 22375951]
92. Kumar S, Rezaei-Ghaleh N, Terwel D, Thal DR, Richard M, Hoch M, Mc Donald JM, Wullner U, Glebov K, Heneka MT, Walsh DM, Zweckstetter M, Walter J. Extracellular phosphorylation of the amyloid beta-peptide promotes formation of toxic aggregates during the pathogenesis of Alzheimer's disease. EMBO J. 2011; 30:2255–2265. [PubMed: 21527912]
93. Kumar S, Wirths O, Theil S, Gerth J, Bayer TA, Walter J. Early intraneuronal accumulation and increased aggregation of phosphorylated Abeta in a mouse model of Alzheimer's disease. Acta Neuropathol. 2013; 125:699–709. [PubMed: 23525537]
94. Milton NG. Phosphorylation of amyloid-beta at the serine 26 residue by human cdc2 kinase. Neuroreport. 2001; 12:3839–3844. [PubMed: 11726805]
95. Milton NG. Phosphorylated amyloid-beta: the toxic intermediate in alzheimer's disease neurodegeneration. Subcell Biochem. 2005; 38:381–402. [PubMed: 15709490]
96. Kaneko I, Morimoto K, Kubo T. Drastic neuronal loss in vivo by beta-amyloid racemized at Ser(26) residue: conversion of non-toxic [D-Ser(26)]beta-amyloid 1-40 to toxic and proteinase-resistant fragments. Neuroscience. 2001; 104:1003–1011. [PubMed: 11457586]
97. Kubo T, Nishimura S, Kumagae Y, Kaneko I. In vivo conversion of racemized beta-amyloid ([D-Ser 26]A beta 1-40) to truncated and toxic fragments ([D-Ser 26]A beta 25–35/40) and fragment presence in the brains of Alzheimer's patients. J Neurosci Res. 2002; 70:474–483. [PubMed: 12391608]
98. Kubo T, Kumagae Y, Miller CA, Kaneko I. Beta-amyloid racemized at the Ser26 residue in the brains of patients with Alzheimer disease: implications in the pathogenesis of Alzheimer disease. J Neuropathol Exp Neurol. 2003; 62:248–259. [PubMed: 12638729]
99. Butterfield DA, Kanski J. Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's amyloid beta-peptide 1-42. Peptides. 2002; 23:1299–1309. [PubMed: 12128086]
100. Ali FE, Separovic F, Barrow CJ, Cherny RA, Fraser F, Bush AI, Masters CL, Barnham KJ. Methionine regulates copper/hydrogen peroxide oxidation products of Abeta. J Pept Sci. 2005; 11:353–360. [PubMed: 15635660]
101. Butterfield DA, Galvan V, Lange MB, Tang H, Sowell RA, Spilman P, Fombonne J, Gorostiza O, Zhang J, Sultana R, Bredesen DE. In vivo oxidative stress in brain of Alzheimer disease transgenic mice: Requirement for methionine 35 in amyloid beta-peptide of APP. Free Radic Biol Med. 2010; 48:136–144. [PubMed: 19854267]
102. Varadarajan S, Yatin S, Kanski J, Jahanshahi F, Butterfield DA. Methionine residue 35 is important in amyloid beta-peptide-associated free radical oxidative stress. Brain Res Bull. 1999; 50:133–141. [PubMed: 10535332]
103. Moskovitz J, Maiti P, Lopes DH, Oien DB, Attar A, Liu T, Mittal S, Hayes J, Bitan G. Induction of methionine-sulfoxide reductases protects neurons from amyloid beta-protein insults in vitro and in vivo. Biochemistry. 2011; 50:10687–10697. [PubMed: 22059533]
104. Hou L, Kang I, Marchant RE, Zagorski MG. Methionine 35 oxidation reduces fibril assembly of the amyloid abeta-(1-42) peptide of Alzheimer's disease. J Biol Chem. 2002; 277:40173–40176. [PubMed: 12198111]
105. Hou L, Lee HG, Han F, Tedesco JM, Perry G, Smith MA, Zagorski MG. Modification of amyloid-beta1-42 fibril structure by methionine-35 oxidation. J Alzheimers Dis. 2013; 37:9–18. [PubMed: 23719512]
106. Narayanan S, Kamps B, Boelens WC, Reif B. alphaB-crystallin competes with Alzheimer's disease beta-amyloid peptide for peptide-peptide interactions and induces oxidation of Abeta-Met35. FEBS Lett. 2006; 580:5941–5946. [PubMed: 17046756]

107. Johansson AS, Bergquist J, Volbracht C, Paivio A, Leist M, Lannfelt L, Westlind-Danielsson A. Attenuated amyloid-beta aggregation and neurotoxicity owing to methionine oxidation. Neuroreport. 2007; 18:559–563. [PubMed: 17413657]

108. Brown AM, Lemkul JA, Schaum N, Bevan DR. Simulations of monomeric amyloid beta-peptide (1-40) with varying solution conditions and oxidation state of Met35: implications for aggregation. Arch Biochem Biophys. 2014; 545:44–52. [PubMed: 24418316]

109. Kummer MP, Hermes M, Delekarte A, Hammerschmidt T, Kumar S, Terwel D, Walter J, Pape HC, Konig S, Roeber S, Jessen F, Klockgether T, Korte M, Heneka MT. Nitration of tyrosine 10 critically enhances amyloid beta aggregation and plaque formation. Neuron. 2011; 71:833–844. [PubMed: 21903077]

110. Zhao J, Wang P, Li H, Gao Z. Nitration of Y10 in Abeta1-40: is it a compensatory reaction against oxidative/nitrative stress and Abeta aggregation? Chem Res Toxicol. 2015; 28:401–407. [PubMed: 25387246]

111. Harigaya Y, Shoji M, Kawarabayashi T, Kanai M, Nakamura T, Iizuka T, Igeta Y, Saido TC, Sahara N, Mori H, Hirai S. Modified amyloid beta protein ending at 42 or 40 with different solubility accumulates in the brain of Alzheimer's disease. Biochem Biophys Res Commun. 1995; 211:1015–1022. [PubMed: 7598687]

112. Kuo YM, Emmerling MR, Vigo-Pelfrey C, Kasunic TC, Kirkpatrick JB, Murdoch GH, Ball MJ, Roher AE. Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. J Biol Chem. 1996; 271:4077–4081. [PubMed: 8626743]

113. Roher AE, Chaney MO, Kuo YM, Webster SD, Stine WB, Haverkamp LJ, Woods AS, Cotter RJ, Tuohy JM, Krafft GA, Bonnell BS, Emmerling MR. Morphology and toxicity of Abeta-(1-42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. J Biol Chem. 1996; 271:20631–20635. [PubMed: 8702810]

114. Tabaton M, Nunzi MG, Xue R, Usiak M, Autilio-Gambetti L, Gambetti P. Soluble amyloid beta-protein is a marker of Alzheimer amyloid in brain but not in cerebrospinal fluid. Biochem Biophys Res Commun. 1994; 200:1598–1603. [PubMed: 8185615]

115. Teller JK, Russo C, DeBusk LM, Angelini G, Zaccheo D, Dagna-Bricarelli F, Scartezzini P, Bertolini S, Mann DM, Tabaton M, Gambetti P. Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. Nat Med. 1996; 2:93–95. [PubMed: 8564851]

116. Chaney MO, Webster SD, Kuo YM, Roher AE. Molecular modeling of the Abeta1-42 peptide from Alzheimer's disease. Protein Eng. 1998; 11:761–767. [PubMed: 9796824]

117. Garzon-Rodriguez W, Sepulveda-Becerra M, Milton S, Glabe CG. Soluble amyloid Abeta-(1-40) exists as a stable dimer at low concentrations. J Biol Chem. 1997; 272:21037–21044. [PubMed: 9261105]

118. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science. 2003; 300:486–489. [PubMed: 12702875]

119. Kokubo H, Kayed R, Glabe CG, Yamaguchi H. Soluble Abeta oligomers ultrastructurally localize to cell processes and might be related to synaptic dysfunction in Alzheimer's disease brain. Brain Res. 2005; 1031:222–228. [PubMed: 15649447]

120. Deshpande A, Mina E, Glabe C, Busciglio J. Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons. J Neurosci. 2006; 26:6011–6018. [PubMed: 16738244]

121. Jin M, Shepardson N, Yang T, Chen G, Walsh D, Selkoe DJ. Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. Proc Natl Acad Sci U S A. 2011; 108:5819–5824. [PubMed: 21421841]

122. Watt AD, Perez KA, Rembach A, Sherrat NA, Hung LW, Johanssen T, McLean CA, Kok WM, Hutton CA, Fodero-Tavoletti M, Masters CL, Villemagne VL, Barnham KJ. Oligomers, fact or artefact? SDS-PAGE induces dimerization of beta-amyloid in human brain samples. Acta Neuropathol. 2013; 125:549–564. [PubMed: 23354835]

123. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Amyloid-beta protein

dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008; 14:837–842. [PubMed: 18568035]

124. Hung LW, Ciccotosto GD, Giannakis E, Tew DJ, Perez K, Masters CL, Cappai R, Wade JD, Barnham KJ. Amyloid-beta peptide (Abeta) neurotoxicity is modulated by the rate of peptide aggregation: Abeta dimers and trimers correlate with neurotoxicity. J Neurosci. 2008; 28:11950–11958. [PubMed: 19005060]
125. Hong S, Ostaszewski BL, Yang T, O'Malley TT, Jin M, Yanagisawa K, Li S, Bartels T, Selkoe DJ. Soluble Abeta Oligomers Are Rapidly Sequestered from Brain ISF In Vivo and Bind GM1 Ganglioside on Cellular Membranes. Neuron. 2014; 82:308–319. [PubMed: 24685176]
126. Crouch PJ, Blake R, Duce JA, Ciccotosto GD, Li QX, Barnham KJ, Curtain CC, Cherny RA, Cappai R, Dyrks T, Masters CL, Trounce IA. Copper-dependent inhibition of human cytochrome c oxidase by a dimeric conformer of amyloid-beta1-42. J Neurosci. 2005; 25:672–679. [PubMed: 15659604]
127. Kim BH, Palermo NY, Lovas S, Zaikova T, Keana JF, Lyubchenko YL. Single-molecule atomic force microscopy force spectroscopy study of Abeta-40 interactions. Biochemistry. 2011; 50:5154–5162. [PubMed: 21553928]
128. Lv Z, Roychaudhuri R, Condron MM, Teplow DB, Lyubchenko YL. Mechanism of amyloid beta-protein dimerization determined using single-molecule AFM force spectroscopy. Sci Rep. 2013; 3:2880. [PubMed: 24096987]
129. Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. Trends Neurosci. 2008; 31:454–463. [PubMed: 18675468]
130. Fandrich M. Oligomeric intermediates in amyloid formation: structure determination and mechanisms of toxicity. J Mol Biol. 2012; 421:427–440. [PubMed: 22248587]
131. Larson ME, Lesne SE. Soluble Abeta oligomer production and toxicity. J Neurochem. 2012; 120(Suppl 1):125–139.
132. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci. 2010; 13:812–818. [PubMed: 20581818]
133. Reddy PH. Amyloid beta, mitochondrial structural and functional dynamics in Alzheimer's disease. Exp Neurol. 2009; 218:286–292. [PubMed: 19358844]
134. Yang X, Askarova S, Lee JC. Membrane biophysics and mechanics in Alzheimer's disease. Mol Neurobiol. 2010; 41:138–148. [PubMed: 20437210]
135. Sengupta U, Nilson AN, Kayed R. The Role of Amyloid-beta Oligomers in Toxicity, Propagation, and Immunotherapy. EBioMedicine. 2016; 6:42–49. [PubMed: 27211547]
136. Balducci C, Forloni G. In vivo application of beta amyloid oligomers: a simple tool to evaluate mechanisms of action and new therapeutic approaches. Curr Pharm Des. 2014; 20:2491–2505. [PubMed: 23859553]
137. Brouillette J. The effects of soluble Abeta oligomers on neurodegeneration in Alzheimer's disease. Curr Pharm Des. 2014; 20:2506–2519. [PubMed: 23859546]
138. Klein WL. Synaptotoxic amyloid-beta oligomers: a molecular basis for the cause, diagnosis, and treatment of Alzheimer's disease? J Alzheimers Dis. 2013; 33(Suppl 1):S49–S65. [PubMed: 22785404]
139. Mucke L, Selkoe DJ. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. Cold Spring Harb Perspect Med. 2012; 2:a006338. [PubMed: 22762015]
140. Selkoe DJ. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res. 2008; 192:106–113. [PubMed: 18359102]
141. Wisniewski T, Sigurdsson EM. Murine models of Alzheimer's disease and their use in developing immunotherapies. Biochim Biophys Acta. 2010; 1802:847–859. [PubMed: 20471477]
142. Kiskis J, Fink H, Nyberg L, Thyr J, Li JY, Enejder A. Plaque-associated lipids in Alzheimer's diseased brain tissue visualized by nonlinear microscopy. Sci Rep. 2015; 5:13489. [PubMed: 26311128]
143. Liao L, Cheng D, Wang J, Duong DM, Losik TG, Gearing M, Rees HD, Lah JJ, Levey AI, Peng J. Proteomic characterization of postmortem amyloid plaques isolated by laser capture microdissection. J Biol Chem. 2004; 279:37061–37068. [PubMed: 15220353]

144. Abraham CR, Selkoe DJ, Potter H. Immunochemical identification of the serine protease inhibitor alpha 1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. Cell. 1988; 52:487–501. [PubMed: 3257719]

145. Abraham CR. Reactive astrocytes and alpha1-antichymotrypsin in Alzheimer's disease. Neurobiol Aging. 2001; 22:931–936. [PubMed: 11755001]

146. Hughes TM, Lopez OL, Evans RW, Kamboh MI, Williamson JD, Klunk WE, Mathis CA, Price JC, Cohen AD, Snitz BE, Dekosky ST, Kuller LH. Markers of cholesterol transport are associated with amyloid deposition in the brain. Neurobiol Aging. 2014; 35:802–807. [PubMed: 24199960]

147. Liao F, Zhang TJ, Jiang H, Lefton KB, Robinson GO, Vassar R, Sullivan PM, Holtzman DM. Murine versus human apolipoprotein E4: differential facilitation of and co-localization in cerebral amyloid angiopathy and amyloid plaques in APP transgenic mouse models. Acta Neuropathol Commun. 2015; 3:70. [PubMed: 26556230]

148. Mori T, Paris D, Town T, Rojiani AM, Sparks DL, DelleDonne A, Crawford F, Abdullah LI, Humphrey JA, Dickson DW, Mullan MJ. Cholesterol accumulates in senile plaques of Alzheimer disease patients and in transgenic APP(SW) mice. J Neuropathol Exp Neurol. 2001; 60:778–785. [PubMed: 11487052]

149. Mufson EJ, Benzing WC, Cole GM, Wang H, Emerich DF, Sladek JR Jr, Morrison JH, Kordower JH. Apolipoprotein E-immunoreactivity in aged rhesus monkey cortex: colocalization with amyloid plaques. Neurobiol Aging. 1994; 15:621–627. [PubMed: 7824054]

150. Stewart KL, Hughes E, Yates EA, Akien GR, Huang TY, Lima MA, Rudd TR, Guerrini M, Hung SC, Radford SE, Middleton DA. Atomic Details of the Interactions of Glycosaminoglycans with Amyloid-beta Fibrils. J Am Chem Soc. 2016; 138:8328–8331. [PubMed: 27281497]

151. Xu F, Vitek MP, Colton CA, Previti ML, Gharkholonarehe N, Davis J, Van Nostrand WE. Human apolipoprotein E redistributes fibrillar amyloid deposition in Tg-SwDI mice. J Neurosci. 2008; 28:5312–5320. [PubMed: 18480287]

152. Yanagisawa K. Pathological significance of ganglioside clusters in Alzheimer's disease. J Neurochem. 2011; 116:806–812. [PubMed: 21214549]

153. Hadley KC, Rakhit R, Guo H, Sun Y, Jonkman JE, McLaurin J, Hazrati LN, Emili A, Chakrabartty A. Determining composition of micron-scale protein deposits in neurodegenerative disease by spatially targeted optical microproteomics. Elife. 2015; 4

154. Kimura A, Hata S, Suzuki T. Alternative Selection of beta-Site APP-Cleaving Enzyme 1 (BACE1) Cleavage Sites in Amyloid beta-Protein Precursor (APP) Harboring Protective and Pathogenic Mutations within the Abeta Sequence. J Biol Chem. 2016; 291:24041–24053. [PubMed: 27687728]

155. Gowing E, Roher AE, Woods AS, Cotter RJ, Chaney M, Little SP, Ball MJ. Chemical characterization of A beta 17-42 peptide, a component of diffuse amyloid deposits of Alzheimer disease. J Biol Chem. 1994; 269:10987–10990. [PubMed: 8157623]

156. Higgins LS, Murphy GM Jr, Forno LS, Catalano R, Cordell B. P3 beta-amyloid peptide has a unique and potentially pathogenic immunohistochemical profile in Alzheimer's disease brain. Am J Pathol. 1996; 149:585–596. [PubMed: 8701997]

157. Lalowski M, Golabek A, Lemere CA, Selkoe DJ, Wisniewski HM, Beavis RC, Frangione B, Wisniewski T. The "nonamyloidogenic" p3 fragment (amyloid beta17-42) is a major constituent of Down's syndrome cerebellar preamyloid. J Biol Chem. 1996; 271:33623–33631. [PubMed: 8969231]

158. Yamaguchi H, Nakazato Y, Hirai S, Shoji M, Harigaya Y. Electron micrograph of diffuse plaques. Initial stage of senile plaque formation in the Alzheimer brain. Am J Pathol. 1989; 135:593–597. [PubMed: 2679112]

159. Jang H, Arce FT, Ramachandran S, Capone R, Azimova R, Kagan BL, Nussinov R, Lal R. Truncated beta-amyloid peptide channels provide an alternative mechanism for Alzheimer's Disease and Down syndrome. Proc Natl Acad Sci U S A. 2010; 107:6538–6543. [PubMed: 20308552]

160. Jang H, Connelly L, Arce FT, Ramachandran S, Kagan BL, Lal R, Nussinov R. Mechanisms for the Insertion of Toxic, Fibril-like beta-Amyloid Oligomers into the Membrane. J Chem Theory Comput. 2013; 9:822–833. [PubMed: 23316126]

161. Yu X, Wang Q, Pan Q, Zhou F, Zheng J. Molecular interactions of Alzheimer amyloid-beta oligomers with neutral and negatively charged lipid bilayers. Phys Chem Chem Phys. 2013; 15:8878–8889. [PubMed: 23493873]

162. Xu X. Gamma-secretase catalyzes sequential cleavages of the AbetaPP transmembrane domain. J Alzheimers Dis. 2009; 16:211–224. [PubMed: 19221413]

163. Qi-Takahara Y, Morishima-Kawashima M, Tanimura Y, Dolios G, Hirotani N, Horikoshi Y, Kametani F, Maeda M, Saido TC, Wang R, Ihara Y. Longer forms of amyloid beta protein: implications for the mechanism of intramembrane cleavage by gamma-secretase. J Neurosci. 2005; 25:436–445. [PubMed: 15647487]

164. Takami M, Nagashima Y, Sano Y, Ishihara S, Morishima-Kawashima M, Funamoto S, Ihara Y. gamma-Secretase: successive tripeptide and tetrapeptide release from the transmembrane domain of beta-carboxyl terminal fragment. J Neurosci. 2009; 29:13042–13052. [PubMed: 19828817]

165. Zhao G, Mao G, Tan J, Dong Y, Cui MZ, Kim SH, Xu X. Identification of a new presenilin-dependent zeta-cleavage site within the transmembrane domain of amyloid precursor protein. J Biol Chem. 2004; 279:50647–50650. [PubMed: 15485850]

166. Hartmann T, Bieger SC, Bruhl B, Tienari PJ, Ida N, Allsop D, Roberts GW, Masters CL, Dotti CG, Unsicker K, Beyreuther K. Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides. Nat Med. 1997; 3:1016–1020. [PubMed: 9288729]

167. Selkoe DJ, Wolfe MS. Presenilin: running with scissors in the membrane. Cell. 2007; 131:215–221. [PubMed: 17956719]

168. Wolfe MS. When loss is gain: reduced presenilin proteolytic function leads to increased Abeta42/Abeta40. Talking Point on the role of presenilin mutations in Alzheimer disease. EMBO Rep. 2007; 8:136–140. [PubMed: 17268504]

169. Van Vickle GD, Esh CL, Kokjohn TA, Patton RL, Kalback WM, Luehrs DC, Beach TG, Newel AJ, Lopera F, Ghetti B, Vidal R, Castano EM, Roher AE. Presenilin-1 280Glu–>Ala mutation alters C-terminal APP processing yielding longer abeta peptides: implications for Alzheimer's disease. Mol Med. 2008; 14:184–194. [PubMed: 18317569]

170. Andrew RJ, Kellett KA, Thinakaran G, Hooper NM. A Greek Tragedy: The Growing Complexity of Alzheimer Amyloid Precursor Protein Proteolysis. J Biol Chem. 2016; 291:19235–19244. [PubMed: 27474742]

171. Simons M, De SB, Multhaup G, Tienari PJ, Dotti CG, Beyreuther K. Amyloidogenic processing of the human amyloid precursor protein in primary cultures of rat hippocampal neurons. J Neurosci. 1996; 16:899–908. [PubMed: 8558258]

172. Zhang Z, Song M, Liu X, Su KS, Duong DM, Seyfried NT, Cao X, Cheng L, Sun YE, Ping YS, Jia J, Levey AI, Ye K. Delta-secretase cleaves amyloid precursor protein and regulates the pathogenesis in Alzheimer's disease. Nat Commun. 2015; 6:8762. [PubMed: 26549211]

173. Willem M, Tahirovic S, Busche MA, Ovsepian SV, Chafai M, Kootar S, Hornburg D, Evans LD, Moore S, Daria A, Hampel H, Muller V, Giudici C, Nuscher B, Wenninger-Weinzierl A, Kremmer E, Heneka MT, Thal DR, Giedraitis V, Lannfelt L, Muller U, Livesey FJ, Meissner F, Herms J, Konnerth A, Marie H, Haass C. eta-Secretase processing of APP inhibits neuronal activity in the hippocampus. Nature. 2015; 526:443–447. [PubMed: 26322584]

174. Wang H, Sang N, Zhang C, Raghupathi R, Tanzi RE, Saunders A. Correction to Cathepsin L Mediates the Degradation of Novel APP C-Terminal Fragments. Biochemistry. 2015; 54:5781. [PubMed: 26348101]

175. Berger-Sweeney J, McPhie DL, Arters JA, Greenan J, Oster-Granite ML, Neve RL. Impairments in learning and memory accompanied by neurodegeneration in mice transgenic for the carboxyl-terminus of the amyloid precursor protein. Brain Res Mol Brain Res. 1999; 66:150–162. [PubMed: 10095087]

176. Oster-Granite ML, McPhie DL, Greenan J, Neve RL. Age-dependent neuronal and synaptic degeneration in mice transgenic for the C terminus of the amyloid precursor protein. J Neurosci. 1996; 16:6732–6741. [PubMed: 8824314]

177. Bertrand E, Brouillet E, Caille I, Bouillot C, Cole GM, Prochiantz A, Allinquant B. A short cytoplasmic domain of the amyloid precursor protein induces apoptosis in vitro and in vivo. Mol Cell Neurosci. 2001; 18:503–511. [PubMed: 11922141]

178. Lu DC, Rabizadeh S, Chandra S, Shayya RF, Ellerby LM, Ye X, Salvesen GS, Koo EH, Bredesen DE. A second cytotoxic proteolytic peptide derived from amyloid beta-protein precursor. Nat Med. 2000; 6:397–404. [PubMed: 10742146]

179. Madeira A, Pommet JM, Prochiantz A, Allinquant B. SET protein (TAF1beta, I2PP2A) is involved in neuronal apoptosis induced by an amyloid precursor protein cytoplasmic subdomain. FASEB J. 2005; 19:1905–1907. [PubMed: 16162853]

180. Park SA, Shaked GM, Bredesen DE, Koo EH. Mechanism of cytotoxicity mediated by the C31 fragment of the amyloid precursor protein. Biochem Biophys Res Commun. 2009; 388:450–455. [PubMed: 19679105]

181. Nikolaev A, McLaughlin T, O'Leary DD, Tessier-Lavigne M. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature. 2009; 457:981–989. [PubMed: 19225519]

182. Mattsson N, Insel PS, Palmqvist S, Stomrud E, van WD, Minthon L, Zetterberg H, Blennow K, Hansson O. Increased amyloidogenic APP processing in APOE varepsilon4-negative individuals with cerebral beta-amyloidosis. Nat Commun. 2016; 7:10918. [PubMed: 26948379]

183. Ferrer I, Boada RM, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. Brain Pathol. 2004; 14:11–20. [PubMed: 14997933]

184. Masliah E, Hansen L, Adame A, Crews L, Bard F, Lee C, Seubert P, Games D, Kirby L, Schenk D. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. Neurology. 2005; 64:129–131. [PubMed: 15642916]

185. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med. 2003; 9:448–452. [PubMed: 12640446]

186. Nicoll JA, Barton E, Boche D, Neal JW, Ferrer I, Thompson P, Vlachouli C, Wilkinson D, Bayer A, Games D, Seubert P, Schenk D, Holmes C. Abeta species removal after abeta42 immunization. J Neuropathol Exp Neurol. 2006; 65:1040–1048. [PubMed: 17086100]

187. Patton RL, Kalback WM, Esh CL, Kokjohn TA, Van Vickle GD, Luehrs DC, Kuo YM, Lopez J, Brune D, Ferrer I, Masliah E, Newel AJ, Beach TG, Castano EM, Roher AE. Amyloid-beta peptide remnants in AN-1792-immunized Alzheimer's disease patients: a biochemical analysis. Am J Pathol. 2006; 169:1048–1063. [PubMed: 16936277]

188. Maarouf CL, Daugs ID, Kokjohn TA, Kalback WM, Patton RL, Luehrs DC, Masliah E, Nicoll JA, Sabbagh MN, Beach TG, Castano EM, Roher AE. The biochemical aftermath of anti-amyloid immunotherapy. Mol Neurodegener. 2010; 5:39. [PubMed: 20929585]

189. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet. 2008; 372:216–223. [PubMed: 18640458]

190. Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe DJ. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. J Biol Chem. 1996; 271:32916–32922. [PubMed: 8955133]

191. Bohrmann B, Tjernberg L, Kuner P, Poli S, Levet-Trafit B, Naslund J, Richards G, Huber W, Dobeli H, Nordstedt C. Endogenous proteins controlling amyloid beta-peptide polymerization. Possible implications for beta-amyloid formation in the central nervous system and in peripheral tissues. J Biol Chem. 1999; 274:15990–15995. [PubMed: 10347147]

192. Kuo YM, Kokjohn TA, Kalback W, Luehrs D, Galasko DR, Chevallier N, Koo EH, Emmerling MR, Roher AE. Amyloid-beta peptides interact with plasma proteins and erythrocytes: implications for their quantitation in plasma. Biochem Biophys Res Commun. 2000; 268:750–756. [PubMed: 10679277]

193. Roher AE, Esh CL, Kokjohn TA, Castano EM, Van Vickle GD, Kalback WM, Patton RL, Luehrs DC, Daugs ID, Kuo YM, Emmerling MR, Soares H, Quinn JF, Kaye J, Connor DJ, Silverberg

NB, Adler CH, Seward JD, Beach TG, Sabbagh MN. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. Alzheimers Dement. 2009; 5:18–29. [PubMed: 19118806]

194. Kuo YM, Kokjohn TA, Watson MD, Woods AS, Cotter RJ, Sue LI, Kalback WM, Emmerling MR, Beach TG, Roher AE. Elevated abeta42 in skeletal muscle of Alzheimer disease patients suggests peripheral alterations of AbetaPP metabolism. Am J Pathol. 2000; 156:797–805. [PubMed: 10702395]
195. Kokjohn TA, Van Vickle GD, Maarouf CL, Kalback WM, Hunter JM, Daugs ID, Luehrs DC, Lopez J, Brune D, Sue LI, Beach TG, Castano EM, Roher AE. Chemical characterization of pro-inflammatory amyloid-beta peptides in human atherosclerotic lesions and platelets. Biochim Biophys Acta. 2011; 1812:1508–1514. [PubMed: 21784149]
196. Harper JD, Wong SS, Lieber CM, Lansbury PT. Observation of metastable Abeta amyloid protofibrils by atomic force microscopy. Chem Biol. 1997; 4:119–125. [PubMed: 9190286]
197. Koffie RM, Meyer-Luehmann M, Hashimoto T, Adams KW, Mielke ML, Garcia-Alloza M, Micheva KD, Smith SJ, Kim ML, Lee VM, Hyman BT, Spires-Jones TL. Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. Proc Natl Acad Sci U S A. 2009; 106:4012–4017. [PubMed: 19228947]
198. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci U S A. 1998; 95:6448–6453. [PubMed: 9600986]
199. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol. 1999; 155:853–862. [PubMed: 10487842]
200. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann Neurol. 1999; 46:860–866. [PubMed: 10589538]
201. Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek GB, Selkoe DJ, Teplow DB. Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J Biol Chem. 1999; 274:25945–25952. [PubMed: 10464339]
202. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature. 2002; 416:535–539. [PubMed: 11932745]
203. Wang HW, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, Viola KL, Klein WL, Stine WB, Krafft GA, Trommer BL. Soluble oligomers of beta amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. Brain Res. 2002; 924:133–140. [PubMed: 11750898]
204. Ghiso J, Shayo M, Calero M, Ng D, Tomidokoro Y, Gandy S, Rostagno A, Frangione B. Systemic catabolism of Alzheimer's Abeta40 and Abeta42. J Biol Chem. 2004; 279:45897–45908. [PubMed: 15322125]
205. Miners JS, Baig S, Palmer J, Palmer LE, Kehoe PG, Love S. Abeta-degrading enzymes in Alzheimer's disease. Brain Pathol. 2008; 18:240–252. [PubMed: 18363935]
206. Saito S, Ihara M. New therapeutic approaches for Alzheimer's disease and cerebral amyloid angiopathy. Front Aging Neurosci. 2014; 6:290. [PubMed: 25368578]
207. Bushman DM, Kaeser GE, Siddoway B, Westra JW, Rivera RR, Rehen SK, Yung YC, Chun J. Genomic mosaicism with increased amyloid precursor protein (APP) gene copy number in single neurons from sporadic Alzheimer's disease brains. Elife. 2015; 4
208. Lodato MA, Woodworth MB, Lee S, Evrony GD, Mehta BK, Karger A, Lee S, Chittenden TW, D'Gama AM, Cai X, Luquette LJ, Lee E, Park PJ, Walsh CA. Somatic mutation in single human neurons tracks developmental and transcriptional history. Science. 2015; 350:94–98. [PubMed: 26430121]
209. Klein WL, Stine WB Jr, Teplow DB. Small assemblies of unmodified amyloid beta-protein are the proximate neurotoxin in Alzheimer's disease. Neurobiol Aging. 2004; 25:569–580. [PubMed: 15172732]

210. Lacor PN, Buniel MC, Chang L, Fernandez SJ, Gong Y, Viola KL, Lambert MP, Velasco PT, Bigio EH, Finch CE, Krafft GA, Klein WL. Synaptic targeting by Alzheimer's-related amyloid beta oligomers. J Neurosci. 2004; 24:10191–10200. [PubMed: 15537891]
211. Lee EB, Leng LZ, Zhang B, Kwong L, Trojanowski JQ, Abel T, Lee VM. Targeting amyloid-beta peptide (Abeta) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Abeta precursor protein (APP) transgenic mice. J Biol Chem. 2006; 281:4292–4299. [PubMed: 16361260]
212. Barkhordarian H, Emadi S, Schulz P, Sierks MR. Isolating recombinant antibodies against specific protein morphologies using atomic force microscopy and phage display technologies. Protein Eng Des Sel. 2006; 19:497–502. [PubMed: 16984950]
213. Emadi S, Liu R, Yuan B, Schulz P, McAllister C, Lyubchenko Y, Messer A, Sierks MR. Inhibiting aggregation of alpha-synuclein with human single chain antibody fragments. Biochemistry. 2004; 43:2871–2878. [PubMed: 15005622]
214. Emadi S, Barkhordarian H, Wang MS, Schulz P, Sierks MR. Isolation of a human single chain antibody fragment against oligomeric alpha-synuclein that inhibits aggregation and prevents alpha-synuclein-induced toxicity. J Mol Biol. 2007; 368:1132–1144. [PubMed: 17391701]
215. Emadi S, Kasturirangan S, Wang MS, Schulz P, Sierks MR. Detecting morphologically distinct oligomeric forms of alpha-synuclein. J Biol Chem. 2009; 284:11048–11058. [PubMed: 19141614]
216. Kasturirangan S, Li L, Emadi S, Boddapati S, Schulz P, Sierks MR. Nanobody specific for oligomeric beta-amyloid stabilizes nontoxic form. Neurobiol Aging. 2012; 33:1320–1328. [PubMed: 21067847]
217. Kasturirangan S, Reasoner T, Schulz P, Boddapati S, Emadi S, Valla J, Sierks MR. Isolation and characterization of antibody fragments selective for specific protein morphologies from nanogram antigen samples. Biotechnol Prog. 2013; 29:463–471. [PubMed: 23359572]
218. Liu R, Yuan B, Emadi S, Zameer A, Schulz P, McAllister C, Lyubchenko Y, Goud G, Sierks MR. Single chain variable fragments against beta-amyloid (Abeta) can inhibit Abeta aggregation and prevent abeta-induced neurotoxicity. Biochemistry. 2004; 43:6959–6967. [PubMed: 15170333]
219. Zameer A, Schulz P, Wang MS, Sierks MR. Single chain Fv antibodies against the 25-35 Abeta fragment inhibit aggregation and toxicity of Abeta42. Biochemistry. 2006; 45:11532–11539. [PubMed: 16981713]
220. Zameer A, Kasturirangan S, Emadi S, Nimmagadda SV, Sierks MR. Anti-oligomeric Abeta single-chain variable domain antibody blocks Abeta-induced toxicity against human neuroblastoma cells. J Mol Biol. 2008; 384:917–928. [PubMed: 18929576]
221. Zhou C, Emadi S, Sierks MR, Messer A. A human single-chain Fv intrabody blocks aberrant cellular effects of overexpressed alpha-synuclein. Mol Ther. 2004; 10:1023–1031. [PubMed: 15564134]
222. Kasturirangan S, Boddapati S, Sierks MR. Engineered proteolytic nanobodies reduce Abeta burden and ameliorate Abeta-induced cytotoxicity. Biochemistry. 2010; 49:4501–4508. [PubMed: 20429609]
223. Sierks MR, Chatterjee G, McGraw C, Kasturirangan S, Schulz P, Prasad S. CSF levels of oligomeric alpha-synuclein and beta-amyloid as biomarkers for neurodegenerative disease. Integr Biol (Camb). 2011; 3:1188–1196. [PubMed: 22076255]
224. Williams SM, Schulz P, Sierks MR. Oligomeric alpha-synuclein and beta-amyloid variants as potential biomarkers for Parkinson's and Alzheimer's diseases. Eur J Neurosci. 2016; 43:3–16. [PubMed: 26332448]
225. Watts JC, Condello C, Stohr J, Oehler A, Lee J, DeArmond SJ, Lannfelt L, Ingelsson M, Giles K, Prusiner SB. Serial propagation of distinct strains of Abeta prions from Alzheimer's disease patients. Proc Natl Acad Sci U S A. 2014; 111:10323–10328. [PubMed: 24982139]
226. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005–2010. J Neuropathol Exp Neurol. 2012; 71:266–273. [PubMed: 22437338]
227. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS, Petersen RC, Scheltens P, Sperling RA, Dubois B. A/T/N: An

unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016; 87:539–547. [PubMed: 27371494]

228. Serrano GE, Intorcia A, Carew J, Chiarolanza G, Hidalgo JA, Sue LI, Dugger BN, Saxon-Labelle M, Filon J, Scroggins A, Pullen J, Fornwalt BE, Scott S, Sabbagh MN, Adler CH, Akiyama H, Beach TG. Feasibility Study: Comparison of Frontal Cortex Needle Core Versus Open Biopsy for Detection of Characteristic Proteinopathies of Neurodegenerative Diseases. J Neuropathol Exp Neurol. 2015; 74:934–942. [PubMed: 26230581]

Highlights

- Our work is a critical review of the current state of knowledge regarding the structural and biochemical complexity of AD amyloid.
- We systematically examine several Aβ post translational modifications and other molecular alternatives observed in sporadic AD amyloid and explore their relationships to species present in genetically-mediated familial AD.
- In these review we suggest a mechanism for the characteristic pathogenesis of sporadic AD and have important implications for attempts to stage and mitigate dementia.