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Three Dimensions of the Amyloid Hypothesis: Time, Space, and “Wingmen”

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Abstract

The Amyloid Hypothesis, which has been the predominant framework for research in Alzheimer's Disease (AD) over the past two decades, has also been the source of considerable controversy within the field. The Amyloid Hypothesis postulates that amyloid-beta peptide (A β) is the causative agent in AD, and is strongly supported by data from rare autosomal dominant forms of AD. However, the evidence that A β causes age-associated sporadic AD is more complex and less clear, prompting criticism of the hypothesis. Herein, we provide an overview of the major arguments for and against the Amyloid Hypothesis, and review key data supporting or refuting these arguments. We conclude that A β likely is the key initiator of a complex pathogenic cascade which causes AD, thus supporting the Amyloid Hypothesis in general. However, we argue that A β acts primarily as a trigger of other downstream processes, in particular tau aggregation, which mediate neurodegeneration. Thus, A β appears to be necessary but not sufficient to cause AD, and its major pathogenic effects may occur very early in the disease process. We discuss implications for therapeutic development and future research.

Keywords

amyloid hypothesis; A β ; tau; neurodegeneration; Alzheimer Disease

Introduction

In their original edition of the amyloid cascade hypothesis in 1992, Hardy and Higgins postulated that “amyloid-beta protein (A β)...is the causative agent in Alzheimer's Disease (AD) pathology and that neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a direct result of this deposition”¹. While two decades of research have revealed many layers of complexity, we will argue that the bulk of data still supports a role for A β as the primary initiator of AD pathogenesis. Herein, we will discuss data which supports that amyloid hypothesis, address some key arguments against the amyloid hypothesis, and

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present an updated framework for AD pathogenesis which attempts to reconcile the existing data. It has become clear that A β does not exert its effects in a vacuum, and that a complex network of pathologic processes converge to produce the neuropathologic and clinical syndrome that is AD. However, multiple lines of evidence still support the concept that A β aggregation plays a unique and critical role as the key initiator of AD pathology. By our interpretation of the existing data, A β appears to be necessary but not sufficient for AD, while it may be necessary and sufficient for cerebral amyloid angiopathy (CAA). This argument over the role of A β has important implications regarding the development of therapies, the allocation of research funding and the focus of public and private research efforts.

Genetic factors which increase A β aggregation cause AD

The strongest data supporting the role of A β as disease initiator come from human genetics. Autosomal dominant familial AD (fAD) is caused by mutations in three genes, *APP*, *PSEN1*, and *PSEN2*, which are all integrally involved in A β production². *APP* encodes the Amyloid Precursor Protein, which is the precursor to A β , while *PSEN1* and *PSEN2* encode Presenilin 1 and 2, catalytic subunits of the γ -secretase complex which cleaves APP to generate A β . FAD mutations lead to accelerated accumulation of A β plaques and cause early-onset dementia, cerebral amyloid angiopathy (CAA), or both diseases²⁻⁶. Duplication of the *APP* locus on chromosome 21 increases A β production through increased gene dosage and causes age-related dementia with brain parenchymal A β deposits and CAA⁷⁻⁹. Down syndrome patients, who have trisomy 21 and thus have an extra copy of *APP*, develop age-related amyloid plaque deposition and dementia in an APP-dependent manner¹⁰. In general, pathogenic mutations in all three genes are specifically clustered in regions which serve to increase the level of amyloidogenic APP cleavage by β - or γ -secretase or increase the relative production of amyloidogenic A β_{1-42} versus shorter A β species^{2, 11-13}. Importantly, several APP mutations which reside in the middle of the A β coding region have been identified which cause fAD or CAA by increasing the aggregation propensity or inhibiting degradation of the A β peptide and do not affect A β production^{5, 6}. This suggests that it is A β aggregation, rather than some other alteration in APP processing or presenilin function, which instigates fAD. Thus, fAD provides an opportunity to examine a pure A β -driven disease in relatively young, often healthy patients. In keeping with the amyloid hypothesis, fAD patients first develop fibrillar amyloid plaques, then subsequently accumulate neurofibrillary tau pathology, neuronal loss, and dementia^{14, 15}. Thus, it is relatively easy to argue that the amyloid hypothesis, or a variant of it, is generally true for fAD. Interestingly, fAD-linked mutations clearly impact age-of-onset, but do not strongly affect rate of progression of symptomatic disease. While some of these mutations cause large increases in the production of A β , they do not significantly accelerate progression, suggesting that A β serves as an initiator or pathology, but not a major mediator of downstream neurodegeneration¹⁶.

Fortunately, fAD is extremely rare, and the vast majority of AD cases are considered sporadic (sAD). The genetics of sAD are more complex, and the strongest genetic risk factor is *APOE*. The *APOE* ϵ 4 allele increases risk for AD and CAA, and the ϵ 2 allele decreases risk for AD relative to the ϵ 3 allele^{17, 18}. Approximately 20–25% of the population carries

at least one copy of ApoE4, which increases the risk of AD by ~4 fold (as compared to those with the more common ApoE3/E3 genotype), while 2% of the population carries two E4 alleles, imparting an ~12 fold increased risk¹⁹. While ApoE4 appears to exert a variety of effects in the brain, it is a strong modulator of A β pathology, and knockin mice expressing human ApoE4 along with FAD-linked APP and PSEN1 transgenes have greatly increased A β plaque pathology and reduced A β clearance^{19, 20} while human ApoE2 expressing mice have decreased A β plaque pathology²¹. Numerous studies have demonstrated accelerated amyloid plaque accumulation in human ApoE4 carriers, while preclinical studies show that ApoE influences A β metabolism, with ApoE4 promoting amyloid aggregation and deposition^{20–25}. Reducing ApoE levels, either genetically or with antibody therapy, also reduces A β plaque burden in mice^{26–29}. Furthermore, human ApoE4 is associated with pathogenic changes in CSF A β ₄₂ in cognitively-normal subjects, but not changes in tau, again suggesting that ApoE4 exerts its effects in AD by modulating A β upstream of tau^{23, 30}. Thus, the predominant genetic risk factor for sAD clearly exacerbates A β aggregation. Recently, a rare protective mutation in the APP gene, which diminishes amyloidogenic A β production, has been identified in humans and linked to a decreased risk of developing AD³¹, providing another strong genetic link between A β and sAD.

A β aggregation triggers tau pathology and neurodegeneration in an anatomically discordant manner

One key argument against the amyloid hypothesis is based on the poor correlation, both temporally and anatomically, between A β plaque deposition, neuronal death, and clinical symptoms in sAD. Neuroanatomically, fibrillar A β deposition occurs first and most severely in regions such as the precuneus and frontal lobes, while neuronal death begins and occurs most readily in the entorhinal cortex and hippocampus, regions with relatively few A β plaques^{32, 33}. Tau pathology correlates much more closely with neuronal loss, both spatially and temporally, than amyloid plaques^{33–36}. According to the work of Braak and others, A β pathology appears to begin in the cortex and spread inward, while tau pathology exhibits an opposite progression^{32, 37}. This has prompted critics to suggest that A β must not mediate neurodegeneration in AD and is merely a “bystander”. If this were true, one would expect that in fAD, in which disease pathogenesis is more clearly driven by A β , that there would be a stronger anatomical correlation between plaque deposition and neuronal loss. This is not the case, as fAD pathology closely resembles that of sAD, with A β plaques anatomically disconnected from areas of severe neuronal loss^{14, 15}. Thus, genetic data again demonstrates that it is possible for A β to drive tau pathology and neuronal loss without inherently obvious anatomic colocalization between plaques and areas of neurodegeneration. This anatomic disconnect between fibrillar A β plaques, neurofibrillary tangles, and neuronal loss is still not fully explained, but appears to be consistent between fAD and sAD.

In sAD, the relationship between A β and tau pathology is complex but ultimately supportive of the amyloid hypothesis. Aggregated, phosphorylated tau pathology is present in the brainstem and entorhinal cortex of young, normal, asymptomatic people and precedes the onset of amyloid accumulation³⁸. With age, hippocampal neurofibrillary tau pathology is nearly ubiquitous but remains confined to limbic regions in cognitively-normal, amyloid-

free individuals^{39,40}. However, tau appears to spread into neocortical regions almost solely in people with coexistent A β pathology^{39,41}. These individuals generally go on to develop AD dementia. In several studies examining the cognitively-normal elderly, or patients with mild cognitive impairment or AD widespread cortical tau pathology (Braak stage 3) was commonly observed in patients with concomitant A β plaques, it was never observed in those without plaques, suggesting that the presence of A β aggregation is required for the appearance of high-grade cortical tau pathology^{39,41,42}. Furthermore, while hippocampal tau phosphorylation is commonly observed in cognitively-normal adults, studies show no evidence of neuronal loss in these regions with normal aging^{43,44}. However, neurodegeneration does occur prominently in these regions in AD patients with amyloid plaques^{43,44}, suggesting that tau-mediated toxicity again requires some trigger from A β . Accordingly, “neuritic plaques”, which are amyloid plaques with associated neurofibrillary tau pathology, correlate more closely with neuronal loss and dementia in AD than either plaques or tangles alone⁴⁵. These neuropathologic observations support the idea that while fibrillar A β and neurofibrillary tau pathologies are anatomically separated, A β aggregation appears to somehow result in accelerated neurofibrillary tau pathology and neuronal toxicity. As noted above, fAD mutations, which directly influence A β aggregation, cause tau pathology which is similar to that seen in sAD and correlates closely with neuronal death. However, while mutations in the gene encoding tau (*Mapt*) cause neurodegenerative disease, they do not induce A β pathology^{46,47}. Accordingly, polymorphisms in *Mapt* are associated with increased CSF phospho-tau levels, but only in patients with CSF evidence of A β pathology⁴⁸. Thus, human neuropathological data supports a key tenet of the amyloid hypothesis: that A β triggers or exacerbates downstream tau pathology, though it appears to do so without anatomic colocalization.

Preclinical data from cell culture and animal experiments also support A β as the initiator of tau pathology and downstream neuronal injury. Exposure of primary mouse neuronal cultures to aggregated A β species, derived either from synthetic A β or isolated from human brain, induces tau phosphorylation, neuritic damage, and dendritic spine loss^{49,50}. Interestingly, tau knockout neurons were resistant to neuritic degeneration induced by synthetic or human-derived A β species, while tau overexpression exacerbated A β -induced damage^{51,52}. It is notable that a developmental effect of tau deletion cannot be excluded in these and other experiments, as experiments using post-natal tau deletion have not been reported. In human neurons grown in a matrigel culture system, expression of fAD mutation-bearing APP elicits extracellular A β aggregation and causes subsequent tau pathology⁵³. This tau pathology could be blocked with β - or γ -secretase inhibitors, illustrating the dependence on APP cleavage. In mice, coexpression of mutant human *App* and *Mapt* caused increased severity and spread of neurofibrillary tau pathology, but did not alter A β pathology, again demonstrating that A β aggregation appears upstream of tau^{54,55}. Accordingly, injection of synthetic A β fibrils into the brain of mutant human tau-expressing mice leads to markedly increased tau pathology⁵⁶. A β also impairs axonal transport in cultured neurons in a tau-dependent manner, while behavioral deficits observed in human APP expressing mice were alleviated by tau deletion. Again in these experiments, tau had no impact on A β pathology^{57,58}. In total, substantial preclinical data demonstrates that A β species can directly trigger tau pathology in cells and mice, while human neuropathologic

data supports a model in which tau pathology does not spread into the neocortex until after significant A β pathology has developed. A β aggregation appears to somehow be triggering an exacerbation of tauopathy, which may in turn cause neuronal dysfunction and death. While A β and tau pathology are anatomically separate in the AD brain, considerable evidence demonstrates that A β can still trigger tau pathology and neurodegeneration in regions with minimal fibrillar A β pathology.

Induction of toxic protein misfolding by A β is not isolated to tau. Many individuals with either fAD or sAD also develop α -synuclein aggregation in limbic and cortical regions, suggesting that A β may also accelerate synuclein pathology^{59, 60}. Accordingly, preclinical models demonstrate that A β can induce synuclein pathology^{61, 62}. TDP-43 pathology is also commonly seen in AD patients, and may contribute to neurodegeneration⁶³. Thus, the ability of A β to induce misfolding of other toxic protein, such as tau, synuclein, and TDP-43, may play a critical role in AD pathogenesis, though the exact contribution of some of these protein aggregates is not fully understood.

A β aggregation triggers tau pathology and neurodegeneration in a temporally discordant manner

Temporal discrepancy between the appearance of amyloid plaques, tau tangles, neuronal loss, and clinical dementia is another point of frequent contention with the amyloid hypothesis. Many clinically asymptomatic older individuals are known to have significant amyloid plaque pathology, either at autopsy or by PET imaging. Critics claim that this shows that A β does not cause dementia. Again, it should be noted that fAD patients also develop fibrillar amyloid plaque pathology years before symptom onset, but clearly have A β -driven disease¹⁵. Large cross-sectional biomarker studies suggest that amyloid plaque pathology is not asymptomatic, but instead represents preclinical AD, though the pathologic “incubation time” between the appearance of plaques and onset of tau pathology can be several years, and the time to onset of clinical dementia can be over a decade^{64–68}. Emerging longitudinal evidence from several groups supports this concept, as asymptomatic individuals with biomarker evidence solely of amyloid plaques (either by CSF or PET amyloid imaging markers, with no tau or other cell injury markers) are ~4 times more likely than those without plaques to develop clinical dementia within the ensuing 2–7 years, though the length of followup is likely still too short to fully appreciate this risk^{66–71}. If amyloid plaques are present and there is biomarker evidence of brain injury (such as decrease glucose uptake, hippocampal atrophy, or elevated CSF tau), risk of conversion to clinical dementia is even higher, with relative risks exceeding 10^{66, 67, 71, 72}. People who are ostensibly cognitively normal but have amyloid plaques also exhibit very subtle cognitive deficits on detailed neuropsychometric testing and accelerated hippocampal atrophy when compared to plaque-free controls, suggesting that A β deposition may cause a mild but progressive pathological state prior to the onset of more rampant neurodegeneration with tauopathy and synucleinopathy^{73, 74}. While not conclusive evidence of the central role of A β in AD, the bulk of human biomarker data supports the amyloid hypothesis, as A β -related changes precede tau biomarker changes and cognitive markers by years, are associated with mild but progressive neuropathology, and are predictive of subsequent dementia.

How does A β initiate AD pathology?

So how do we reconcile this anatomic and temporal discordance between fibrillar A β pathology, tau aggregation, and neurodegeneration in both fAD and sAD? One explanation is that in addition to fibrillar plaques, oligomeric forms of A β may mediate further pathology in AD. Indeed, a variety of cytotoxic oligomeric A β species have been identified in AD brain lysates which can exert a wide variety of pathogenic effects in vitro and in the mouse brain^{75–77}. These oligomeric A β species do not necessarily correlate temporally or spatially with plaques, and can be purified from brain regions which are subject to intense neuronal loss, such as the hippocampus^{78–80}. Indeed, there is some evidence that the presence of A β oligomers predicts the presence of dementia more accurately than plaque burden^{78, 80}. In humans, A β oligomers appear to accumulate with age, and are correlated with the appearance of tau pathology^{81, 82}. Mice which accumulate A β oligomers but not fibrillar plaques also develop synaptic damage, inflammation, and cognitive impairment^{83, 84}. However, it is difficult to rectify the oligomer hypothesis with the long prodromal phase of human AD during which plaques are evident but significant neurodegeneration is not, or to explain why oligomers would not lead to more significant neuronal loss in animal models of A β deposition. Furthermore, the specific species of A β which mediate downstream pathology remain an open question^{75, 85}. While the direct neurotoxicity of A β oligomers in vivo is unclear, these species have been shown to directly initiate tau phosphorylation both in vitro and in vivo^{52, 83, 86}. At a molecular level, oligomeric A β may initiate tauopathy through specific signaling events in neurons, such as activation of the Src kinase Fyn^{58, 87, 88}, inducing proteases which modify tau⁸⁹, or activating specific tau-targeting kinases⁹⁰, though none of these have been adequately demonstrated in humans. Given increasing evidence that A β , tau and α -synuclein aggregates all have prion-like properties and can potentially seed normal forms of these proteins and spread through the brain, it may also be important to investigate the ability of A β species to exacerbate the prion-like spread of tau and synuclein^{91–96}. Finally, A β could cause large-scale alterations in the brain's ability to maintain proper protein quality control (proteostasis), eventually prompting aggregation of tau, synuclein, and other intracellular proteins and causing toxicity^{97–99}. Proteostatic failure could explain α -synuclein in the brains of fAD patients, many of whom are too young to have accumulated such deposits as a function of age, or coexistent α -synuclein and TDP-43 aggregates in sAD patients⁶³. A β -induced aggregation and subsequent propagation and cross-templating of other toxic proteins could overwhelm proteostatic mechanisms. As many cytoprotective and proteostatic systems in neurons and glial decline with age, aging might thus set the stage for disseminated A β toxicity^{100, 101}. Other age-related effects or comorbid pathologies (such as diabetes, head trauma, oxidative stress, inflammation, vascular factors, or even sleep deprivation) might influence the rate of A β aggregation and accumulation, accelerate the rate at which A β triggers downstream pathology, or directly exacerbate downstream pathology, thereby modulating the onset of dementia.

A β does not operate in a vacuum

A shortcoming of the initial amyloid hypothesis is the suggestion that A β itself could directly cause the panoply of pathologic changes observed in AD. A β critics have frequently

and correctly pointed out the many other destructive phenomena, from oxidative stress to impaired autophagy, which occur in AD brain. Certainly when one uses the clinical endpoint of cognitive impairment, many factors appear to accelerate AD, cerebrovascular disease being foremost among them. The relationship between these factors, A β , and neurodegeneration is unclear. Mice harboring fAD mutations develop neuroinflammation, oxidative stress, mitochondrial dysfunction, and other pathologic alterations which thus appear to be downstream changes which indicate A β -induced neuronal or glial injury, though many of these markers appear before significant plaque deposition^{102–104}. However, preclinical data suggests that neuronal stressors such as oxidative and nitrate stress^{105, 106}, mitochondrial dysfunction¹⁰⁷, and inflammation¹⁰⁸, can also directly influence APP metabolism and A β accumulation, suggesting the existence of a possible feed-forward mechanism by which A β -induced injury can facilitate A β production, or by which other stressors, such as ischemia or trauma, might exacerbate A β production and toxicity.

While it may be tempting to implicate these other processes as the causative factors in AD, it is important to consider that A β plaque pathology is uniquely indicative of the clinical syndrome of AD, while oxidative damage, inflammation, mitochondrial dysfunction, and other pathogenic markers are observed in many neurologic diseases. Furthermore, there is no strong evidence from humans that genetic mutations which increase oxidative stress, inflammation, mitochondrial dysfunction, or inhibit autophagy lead to A β pathology in the brain. Risk factors such as smoking can have A β -dependent and independent effects on AD pathogenesis, as smoking causes systemic oxidative stress, vascular injury, and may increase A β accumulation^{109, 110}. Similarly, polymorphisms in *TREM2* affect microglial activation and increase risk of AD, yet *TREM2* hemizygous mice have no increase in A β plaques, suggesting that microglial dysfunction could influence AD pathogenesis independently of the amount of A β accumulation¹¹¹. In both cases, it is likely that these risk factors influence pathogenic mechanism downstream of A β , and are not unique to AD. Accordingly, smoking increases the risk of many disease of the brain, including vascular dementia, while *TREM2* likely does the same (it has recently been linked to increased risk of ALS, FTD, and Parkinson disease)^{112, 113}. It would be foolish to argue that these many processes are not important in AD, as they clearly contribute to disease pathogenesis and might provide excellent therapeutic targets. But the existence of these other mechanisms of neural injury in AD does not mean they are causative, nor does it negate the central importance of A β .

Another alternative concept of AD pathogenesis suggests that APP cleavage is indeed critical to AD, but that APP fragments other than A β are the pathogenic species. Mice with inducible expression of APP develop A β plaques and cognitive impairment. The cognitive impairment resolved in part when the APP transgene expression was turned off, despite persisting A β plaques¹¹⁴. Conversely, mice which express A β from a truncated transgene (and not full-length APP) accumulate multiple A β species as well as plaques, but do not develop cognitive impairment¹¹⁵. Several studies have implicated the APP β -C-terminal fragment (β -CTF) as a possible mediator of cognitive impairment in APP-overexpressing mice^{116, 117}. However, it should be noted that antibodies targeting A β reduce plaque burden and rescue cognition in APP transgenic mice without eliminating plaques¹¹⁸. Moreover, as mentioned earlier, APP mutations within the A β coding region which enhance A β aggregation without influence APP cleavage still cause human fAD^{5, 6}. Thus, the data

implicating alternative APP fragments in AD is intriguing, it is still largely restricted to mouse models, and does not discount the contribution of A β .

APP is a complex molecule with a variety of neurobiological functions¹¹⁹. Thus, it is important to note that cognitive/behavioral changes in APP-overexpressing mice are not fully correlated with A β pathology, and are likely influenced greatly by both the APP transgene and overexpression promoter. Cognitive impairment in APP mice can appear before, after, or without significant A β deposition, and are generally reversible^{85, 118}. In human AD, however, cognitive decline is notable years or even decades after extensive plaque pathology accumulates, and is almost always associated with the onset of tau pathology and neurodegeneration, and has not been shown to be reversible. Therefore, the nature of cognitive impairment in mouse APP models and humans may be fundamentally different, and mouse results should be interpreted carefully.”

What initiates A β aggregation?

If A β is the initiator of AD pathology, what initially triggers the aggregation and accumulation of A β in sAD? How is this related to age? Currently, we have no iron-clad explanation for why A β initially accumulates and forms plaques in sAD, or why this pathology occurs late in life. A β might accumulate in a concentration-dependent manner throughout life, with increased neuronal activity in certain brain regions leading to excessive A β production, ultimately causing aggregation and seed formation which then propagates^{91, 120}. Factors which enhance A β production or aggregation, or which suppress A β clearance could contribute to this over a lifetime. Impaired sleep-wake cycle, for example, could contribute to gradual A β accumulation by promoting a higher level of neuronal A β release, and by disrupting normal diurnal oscillations in extracellular A β levels¹²¹. Sleep deprivation could also impair the clearance of A β from the brain by bulk fluid movement, or “glymphatic” flow¹²². As another example, age-related oxidative and nitrative stress could also exert gradual influence on A β production by altering secretase function^{105, 106}, and by promoting A β aggregation through oxidative modification of the peptide^{123, 124}. Another possibility is that age-related decline in the clearance of A β causes initial A β accumulation. Glial uptake of A β , as well as the aforementioned “glymphatic” bulk-flow removal of proteins from the brain, both decline with age^{125, 126}. Studies in humans have shown diminished A β clearance in AD patients¹²⁷, though this has not yet been demonstrated to precede plaque deposition. Age-related disturbances in proteostasis and neuronal stress response signaling might also shift the balance of A β metabolism toward aggregation^{100, 101, 128}.

We propose a model in which A β serves as the primary initiator of AD pathogenesis (see Fig. 1). In our model, A β levels exist in a carefully orchestrated homeostasis throughout life, and factors during middle age which disturb that balance facilitate A β aggregation. These factors include increased synaptic activity and sleep disruption, which lead to increase A β release, psychological stress (which can increase A β production via different mechanisms), the function or genotype of proteins (e.g. ApoE, Lrp1, and SorL1) which regulate A β trafficking and clearance, age-related declines in A β clearance mechanisms, and cellular stressors such as reactive oxygen species and ischemia which might facilitate A β production

or aggregation. As A β homeostasis is lost, oligomeric and fibrillar A β species begin to accumulate, and the first plaques appear, providing the first biomarker evidence of AD in humans, though the patient remains clinically asymptomatic. After several years of A β aggregation, A β somehow triggers the acceleration of AD-type tau pathology, as neurofibrillary tangles begin to spread outside the limbic system and into the neocortex, prompting increases in neurodegeneration reflected as an increase in CSF tau and p-tau levels as well as other neuronal markers (e.g. VILIP-1) ⁷². This “tau trigger” event is still poorly understood, and may be mediated by the appearance of particular toxic A β species, the activation an intermediary signaling process or kinase cascade, changes in the innate immune system, or represent a breaking point at which the proteostatic capacity of the brain has been overwhelmed, facilitating tau aggregation and spreading. Aggregation of other toxic proteins such as synuclein and TDP-43 may also begin at this stage. At this point, the cascade is set in full motion, as neuronal loss, oxidative damage, inflammation, and clinical symptoms become evident, and neutralizing or removing A β is unlikely to have a major effect. Age-related factors and comorbid pathologies likely contribute to the occurrence of AD by injuring neurons in parallel with A β and tau, thereby accelerating the appearance of symptoms, though some such factors may directly regulate A β levels or impact the A β -tau interaction. Thus, preventative strategies for AD would focus on treatment of conditions which promote A β accumulation in middle age, such as sleep disorders. Anti-A β therapies would need to be delivered as early in the process as possible, while the appearance of positive A β biomarkers would be most helpful for timing the initiation of therapies targeting downstream pathologies, such as anti-tau agents.

Has the amyloid hypothesis been tested pharmacologically in humans?

Mention of AD therapeutics elicits one of the more recent arguments against the amyloid hypothesis: the failure of clinical trials targeting A β . The possibility that A β initiates pathology very early in the disease suggests that only early anti-A β therapy is likely to be effective, and to date, no completed trials have attempted therapy early in the A β cascade. Further, A β target engagement has not yet been adequate to truly test the amyloid hypothesis. The Elan AN1792 active A β immunization study (which was halted due to several cases of meningoencephalitis) is often cited as an indictment of the amyloid hypothesis, as several remaining patients were followed after immunization and failed to show any clinical benefit despite evidence of reduced plaque burden at autopsy ¹²⁹. However, these patients were immunized late in the course of disease, well after dementia was clinically apparent, and the small sample size in a phase I trial primarily assessing safety which was aborted after only a few vaccinations at most makes it difficult to draw any firm conclusions from this work. If one insists on considering this data, it should be noted that analysis of this same cohort did show a modest decrease in tau pathology, while surviving immunized participants had a slight improvement in functional outcomes, despite late treatment initiation ^{130–132}. Ongoing studies, however, may put the amyloid hypothesis to a much more rigorous test. The Dominantly Inherited Alzheimer Network (DIAN) treatment trial and Alzheimer’s Prevention Initiative (API) should address the role of A β in fAD most directly, as both are designed to test if anti-amyloid therapy in primarily presymptomatic fAD patients can prevent pathologic changes and dementia ^{133, 134}. This

represents the earliest therapy that is currently possible, and employs a patient population with more “pure” A β -driven disease. However, one concerning possibility which arises from the “A β as initiator” hypothesis is that the appearance of fibrillar A β pathology could represent a point in the pathogenic cascade which is already too late for effective anti-A β therapy. Ultimately, prior prevention of A β deposition could prove to be the most effective treatment to target A β but whether such an approach will be tested is unclear. These studies, along with similar trials in sAD such as the A4 trial, may dictate in part, the future of A β -targeted therapies. There are also several ongoing trials in very mild and mild dementia due to AD using either BACE inhibition or anti-A β antibodies that will also be important to evaluate. Considering the therapeutic regimens employed in diseases such as diabetes, coronary atherosclerosis, and cancer, it seems reasonable that a multi-target therapeutic approach will be needed for AD, with the selection of targets dictated by the stage of the pathology. Thus, regardless of the accuracy of the amyloid hypothesis, target elucidation and therapy development for a variety of pathogenic processes in AD, from tau aggregation to microglial activation to mitochondrial dysfunction, is critical.

Conclusions

In the past 22 years, it has become clear that the idea that A β caused AD via a simple, linear model of toxicity is very likely incorrect. Our interpretation of the data places A β not as the primary direct neurotoxin that itself alone causes AD, but rather as the initiator of complex network of pathologic changes in the brain, many of them tau-dependent, which culminates years later in neurodegeneration. To successfully forestall the progression of this cascade, multiple therapies targeting several nodes of the neurodegenerative network may be needed, and the timing of these therapies in the disease progression will likely be critical. Many important questions remain to be answered, among them:

- What instigates A β aggregation in sAD? Are there earlier precipitating events which precede A β aggregation and are related to age or other disease processes? How can we prevent this?
 - How does A β aggregation trigger spread of tau and synuclein pathology in the human brain? Are there specific therapeutic targets to prevent this process?
 - At what point in the cascade is it too late to intervene on A β ? Once A β plaques are apparent by imaging or CSF studies, has A β already triggered the critical downstream cascades?
 - Which downstream pathologies are legitimate therapeutic targets, and which are epiphenomena? Post-mortem AD brain tissue reveals a dizzying array of cellular and biochemical alterations, but which can be successfully targeted to mitigate disease?
- What is the pathogenesis of AD-like dementias without amyloid plaques (such as “suspected non-amyloid pathology” (SNAP), or “tangle-predominant AD”)? Are these new subtypes of dementia, which appear clinically similar to AD, biologically separate entities which will require unique therapeutic approaches? If there are no plaques, should we still call it AD?

The answers to these questions, as well the outcomes of the many anti-A β therapeutic trials currently in progress, should bring us closer to a comprehensive understanding of the role and therapeutic potential of A β in AD, and guide the development of the next generation of AD therapeutic regimens.

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References

1. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. 1992; 256:184–185. [PubMed: 1566067]
2. Bettens K, Sleegers K, Van Broeckhoven C. Genetic insights in Alzheimer's disease. *Lancet Neurol*. 2013; 12:92–104. [PubMed: 23237904]
3. Levy E, et al. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science*. 1990; 248:1124–1126. [PubMed: 2111584]
4. Goate A, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*. 1991; 349:704–706. [PubMed: 1671712]
5. Tsubuki S, Takaki Y, Saido TC. Dutch, Flemish, Italian, and Arctic mutations of APP and resistance of A β to physiologically relevant proteolytic degradation. *Lancet*. 2003; 361:1957–1958. [PubMed: 12801742]
6. Tomiyama T, et al. A new amyloid beta variant favoring oligomerization in Alzheimer's-type dementia. *Ann Neurol*. 2008; 63:377–387. [PubMed: 18300294]
7. Rovelet-Lecrux A, et al. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat Genet*. 2006; 38:24–26. [PubMed: 16369530]
8. Sleegers K, et al. APP duplication is sufficient to cause early onset Alzheimer's dementia with cerebral amyloid angiopathy. *Brain*. 2006; 129:2977–2983. [PubMed: 16921174]
9. Cabrejo L, et al. Phenotype associated with APP duplication in five families. *Brain*. 2006; 129:2966–2976. [PubMed: 16959815]
10. Prasher VP, et al. Molecular mapping of Alzheimer-type dementia in Down's syndrome. *Ann Neurol*. 1998; 43:380–383. [PubMed: 9506555]
11. Citron M, et al. Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature*. 1992; 360:672–674. [PubMed: 1465129]
12. Eckman CB, et al. A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A β 42(43). *Hum Mol Genet*. 1997; 6:2087–2089. [PubMed: 9328472]
13. Chavez-Gutierrez L, et al. The mechanism of gamma-Secretase dysfunction in familial Alzheimer disease. *EMBO J*. 2012; 31:2261–2274. [PubMed: 22505025]
14. Shepherd C, McCann H, Halliday GM. Variations in the neuropathology of familial Alzheimer's disease. *Acta Neuropath*. 2009; 118:37–52. [PubMed: 19306098]
15. Bateman RJ, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med*. 2012; 367:795–804. [PubMed: 22784036]
16. Ryman DC, et al. Symptom onset in autosomal dominant Alzheimer disease: a systematic review and meta-analysis. *Neurology*. 2014; 83:253–260. [PubMed: 24928124]
17. Corder EH, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993; 261:921–923. [PubMed: 8346443]
18. Chiang GC, et al. Hippocampal atrophy rates and CSF biomarkers in elderly APOE2 normal subjects. *Neurology*. 2010; 75:1976–1981. [PubMed: 20980669]
19. Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurol*. 2011; 10:241–252. [PubMed: 21349439]

20. Castellano JM, et al. Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. *Sci Transl Med*. 2011; 3:89ra57.
21. Fagan AM, et al. Human and murine ApoE markedly alters A beta metabolism before and after plaque formation in a mouse model of Alzheimer's disease. *Neurobiol Dis*. 2002; 9:305–318. [PubMed: 11950276]
22. Hudry E, et al. Gene transfer of human Apoe isoforms results in differential modulation of amyloid deposition and neurotoxicity in mouse brain. *Sci Transl Med*. 2013; 5:212ra161.
23. Morris JC, et al. APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Ann Neurol*. 2010; 67:122–131. [PubMed: 20186853]
24. Vemuri P, et al. Effect of apolipoprotein E on biomarkers of amyloid load and neuronal pathology in Alzheimer disease. *Ann Neurol*. 2010; 67:308–316. [PubMed: 20373342]
25. Verghese PB, et al. ApoE influences amyloid-beta (Abeta) clearance despite minimal apoE/Abeta association in physiological conditions. *Proc Natl Acad Sci USA*. 2013; 110:E1807–E1816. [PubMed: 23620513]
26. Bales KR, et al. Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet*. 1997; 17:263–264. [PubMed: 9354781]
27. Bien-Ly N, Gillespie AK, Walker D, Yoon SY, Huang Y. Reducing human apolipoprotein E levels attenuates age-dependent Abeta accumulation in mutant human amyloid precursor protein transgenic mice. *J Neurosci*. 2012; 32:4803–4811. [PubMed: 22492035]
28. Kim J, et al. Haploinsufficiency of human APOE reduces amyloid deposition in a mouse model of amyloid-beta amyloidosis. *J Neurosci*. 2011; 31:18007–18012. [PubMed: 22159114]
29. Kim J, et al. Anti-apoE immunotherapy inhibits amyloid accumulation in a transgenic mouse model of Abeta amyloidosis. *J Exp Med*. 2012; 209:2149–2156. [PubMed: 23129750]
30. Sunderland T, et al. Cerebrospinal fluid beta-amyloid1–42 and tau in control subjects at risk for Alzheimer's disease: the effect of APOE epsilon4 allele. *Biol Psych*. 2004; 56:670–676.
31. Jonsson T, et al. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature*. 2012; 488:96–99. [PubMed: 22801501]
32. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropath*. 1991; 82:239–259. [PubMed: 1759558]
33. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med*. 2011; 1:a006189. [PubMed: 22229116]
34. Gomez-Isla T, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol*. 1997; 41:17–24. [PubMed: 9005861]
35. Arriagada PV, Marzloff K, Hyman BT. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology*. 1992; 42:1681–1688. [PubMed: 1307688]
36. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology*. 1992; 42:631–639. [PubMed: 1549228]
37. Price JL, Davis PB, Morris JC, White DL. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol Aging*. 1991; 12:295–312. [PubMed: 1961359]
38. Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neurolpath*. 2011; 121:171–181.
39. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol*. 1999; 45:358–368. [PubMed: 10072051]
40. Elobeid A, Soininen H, Alafuzoff I. Hyperphosphorylated tau in young and middle-aged subjects. *Acta Neuropath*. 2012; 123:97–104. [PubMed: 22160320]
41. Knopman DS, et al. Neuropathology of cognitively normal elderly. *J Neuropath Exp Neurol*. 2003; 62:1087–1095. [PubMed: 14656067]
42. Petersen RC, et al. Neuropathologic features of amnesic mild cognitive impairment. *Arch Neurol*. 2006; 63:665–672. [PubMed: 16682536]

43. Gomez-Isla T, et al. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci*. 1996; 16:4491–4500. [PubMed: 8699259]
44. West MJ, Coleman PD, Flood DG, Troncoso JC. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet*. 1994; 344:769–772. [PubMed: 7916070]
45. Tiraboschi P, Hansen LA, Thal LJ, Corey-Bloom J. The importance of neuritic plaques and tangles to the development and evolution of AD. *Neurology*. 2004; 62:1984–1989. [PubMed: 15184601]
46. Reed LA, et al. Autosomal dominant dementia with widespread neurofibrillary tangles. *Annals of neurology*. 1997; 42:564–572. [PubMed: 9382467]
47. Lindquist SG, et al. Alzheimer disease-like clinical phenotype in a family with FTDP-17 caused by a MAPT R406W mutation. *Eur J Neurol*. 2008; 15:377–385. [PubMed: 18284428]
48. Kauwe JS, et al. Variation in MAPT is associated with cerebrospinal fluid tau levels in the presence of amyloid-beta deposition. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:8050–8054. [PubMed: 18541914]
49. Ferreira A, Lu Q, Orecchio L, Kosik KS. Selective phosphorylation of adult tau isoforms in mature hippocampal neurons exposed to fibrillar A beta. *Mol Cell Neurosci*. 1997; 9:220–234. [PubMed: 9245504]
50. Zempel H, Thies E, Mandelkow E, Mandelkow EM. Abeta oligomers cause localized Ca(2+) elevation, misrouting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. *J Neurosci*. 2010; 30:11938–11950. [PubMed: 20826658]
51. Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to beta - amyloid-induced neurotoxicity. *Proc Natl Acad Sci USA*. 2002; 99:6364–6369. [PubMed: 11959919]
52. Jin M, et al. Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. *Proc Natl Acad Sci USA*. 2011; 108:5819–5824. [PubMed: 21421841]
53. Choi SH, et al. A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature*. 2014
54. Hurtado DE, et al. A{beta} accelerates the spatiotemporal progression of tau pathology and augments tau amyloidosis in an Alzheimer mouse model. *Am J Pathol*. 2010; 177:1977–1988. [PubMed: 20802182]
55. Lewis J, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science*. 2001; 293:1487–1491. [PubMed: 11520987]
56. Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P3011 tau transgenic mice induced by Abeta 42 fibrils. *Science*. 2001; 293:1491–1495. [PubMed: 11520988]
57. Roberson ED, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science*. 2007; 316:750–754. [PubMed: 17478722]
58. Roberson ED, et al. Amyloid-beta/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. *J Neurosci*. 2010; 31:700–711. [PubMed: 21228179]
59. Lippa CF, et al. Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer's disease patients with mutations in presenilin and amyloid precursor protein genes. *Am J Pathol*. 1998; 153:1365–1370. [PubMed: 9811326]
60. Hashimoto M, Masliah E. Alpha-synuclein in Lewy body disease and Alzheimer's disease. *Brain Pathol*. 1999; 9:707–720. [PubMed: 10517509]
61. Masliah E, et al. beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc Natl Acad Sci USA*. 2001; 98:12245–12250. [PubMed: 11572944]
62. Larson ME, et al. Soluble alpha-synuclein is a novel modulator of Alzheimer's disease pathophysiology. *J Neurosci*. 2012; 32:10253–10266. [PubMed: 22836259]
63. Josephs KA, et al. TDP-43 is a key player in the clinical features associated with Alzheimer's disease. *Acta Neuropath*. 2014; 127:811–824. [PubMed: 24659241]
64. Jack CR Jr, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013; 12:207–216. [PubMed: 23332364]

65. Jack CR Jr, et al. Evidence for ordering of Alzheimer disease biomarkers. *Arch Neurol.* 2011; 68:1526–1535. [PubMed: 21825215]
66. Roe CM, et al. Amyloid imaging and CSF biomarkers in predicting cognitive impairment up to 7.5 years later. *Neurology.* 2013; 80:1784–1791. [PubMed: 23576620]
67. Vos SJ, et al. Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. *Lancet Neurol.* 2013; 12:957–965. [PubMed: 24012374]
68. Villemagne VL, et al. Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurol.* 2013; 12:357–367. [PubMed: 23477989]
69. Chen X, et al. Pittsburgh compound B retention and progression of cognitive status--a meta-analysis. *Eur J Neurol.* 2014; 21:1060–1067. [PubMed: 24612390]
70. Villemagne VL, et al. Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. *Ann Neurol.* 2011; 69:181–192. [PubMed: 21280088]
71. Knopman DS, et al. Short-term clinical outcomes for stages of NIA-AA preclinical Alzheimer disease. *Neurology.* 2012; 78:1576–1582. [PubMed: 22551733]
72. Tarawneh R, et al. Visinin-like protein-1: diagnostic and prognostic biomarker in Alzheimer disease. *Ann Neurol.* 2011; 70:274–285. [PubMed: 21823155]
73. Donohue MC, et al. The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. *JAMA Neurol.* 2014; 71:961–970. [PubMed: 24886908]
74. Chetelat G, et al. Accelerated cortical atrophy in cognitively normal elderly with high beta-amyloid deposition. *Neurology.* 2012; 78:477–484. [PubMed: 22302548]
75. Shankar GM, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med.* 2008; 14:837–842. [PubMed: 18568035]
76. Walsh DM, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature.* 2002; 416:535–539. [PubMed: 11932745]
77. Mucke L, Selkoe DJ. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med.* 2012; 2:a006338. [PubMed: 22762015]
78. Esparza TJ, et al. Amyloid-beta oligomerization in Alzheimer dementia versus high-pathology controls. *Ann Neurol.* 2013; 73:104–119. [PubMed: 23225543]
79. Tomic JL, Pensalfini A, Head E, Glabe CG. Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction. *Neurobiol Dis.* 2009; 35:352–358. [PubMed: 19523517]
80. McLean CA, et al. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol.* 1999; 46:860–866. [PubMed: 10589538]
81. Lesne SE, et al. Brain amyloid-beta oligomers in ageing and Alzheimer's disease. *Brain.* 2013; 136:1383–1398. [PubMed: 23576130]
82. Handoko M, et al. Correlation of specific amyloid-beta oligomers with tau in cerebrospinal fluid from cognitively normal older adults. *JAMA Neurol.* 2013; 70:594–599. [PubMed: 23479202]
83. Zhang Y, et al. A lifespan observation of a novel mouse model: in vivo evidence supports abeta oligomer hypothesis. *PloS One.* 2014; 9:e85885. [PubMed: 24465766]
84. Tomiyama T, et al. A mouse model of amyloid beta oligomers: their contribution to synaptic alteration, abnormal tau phosphorylation, glial activation, and neuronal loss in vivo. *J Neurosci.* 2010; 30:4845–4856. [PubMed: 20371804]
85. Lesne S, et al. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature.* 2006; 440:352–357. [PubMed: 16541076]
86. Ma QL, et al. Beta-amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling: suppression by omega-3 fatty acids and curcumin. *J Neurosci.* 2009; 29:9078–9089. [PubMed: 19605645]
87. Ittner LM, et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell.* 2010; 142:387–397. [PubMed: 20655099]
88. Haass C, Mandelkow E. Fyn-tau-amyloid: a toxic triad. *Cell.* 2010; 142:356–358. [PubMed: 20691893]

89. Zhang Z, et al. Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease. *Nat Med*. 2014; 20:1254–1262. [PubMed: 25326800]
90. Martin L, et al. Tau protein kinases: involvement in Alzheimer's disease. *Ageing Res Rev*. 2013; 12:289–309. [PubMed: 22742992]
91. Meyer-Luehmann M, et al. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science*. 2006; 313:1781–1784. [PubMed: 16990547]
92. Sanders DW, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron*. 2014; 82:1271–1288. [PubMed: 24857020]
93. de Calignon A, et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron*. 2012; 73:685–697. [PubMed: 22365544]
94. Liu L, et al. Trans-synaptic spread of tau pathology in vivo. *PLoS One*. 2012; 7:e31302. [PubMed: 22312444]
95. Guo JL, et al. Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. *Cell*. 2013; 154:103–117. [PubMed: 23827677]
96. Giasson BI, et al. Initiation and synergistic fibrillization of tau and alpha-synuclein. *Science*. 2003; 300:636–640. [PubMed: 12714745]
97. Dasuri K, Zhang L, Keller JN. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. *Free Radic Biol Med*. 2013; 62:170–185. [PubMed: 23000246]
98. De Strooper B. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. *Physiol Rev*. 2010; 90:465–494. [PubMed: 20393191]
99. Nixon RA, Yang DS. Autophagy failure in Alzheimer's disease--locating the primary defect. *Neurobiol Dis*. 2011; 43:38–45. [PubMed: 21296668]
100. Taylor RC, Dillin A. Aging as an event of proteostasis collapse. *Cold Spring Harb Perspect Biol*. 2011; 3
101. Lu T, et al. REST and stress resistance in ageing and Alzheimer's disease. *Nature*. 2014; 507:448–454. [PubMed: 24670762]
102. Pratico D, Uryu K, Leight S, Trojanowski JQ, Lee VM. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J Neurosci*. 2001; 21:4183–4187. [PubMed: 11404403]
103. Benzing WC, et al. Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. *Neurobiol Aging*. 1999; 20:581–589. [PubMed: 10674423]
104. Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Hum Mol Genet*. 2011; 20:4515–4529. [PubMed: 21873260]
105. Guix FX, et al. Modification of gamma-secretase by nitrosative stress links neuronal ageing to sporadic Alzheimer's disease. *EMBO Mol Med*. 2012; 4:660–673. [PubMed: 22488900]
106. Wahlster L, et al. Presenilin-1 adopts pathogenic conformation in normal aging and in sporadic Alzheimer's disease. *Acta Neuropath*. 2013; 125:187–199. [PubMed: 23138650]
107. Kukreja L, Kujoth GC, Prolla TA, Van Leuven F, Vassar R. Increased mtDNA mutations with aging promotes amyloid accumulation and brain atrophy in the APP/Ld transgenic mouse model of Alzheimer's disease. *Mol Neurodegener*. 2014; 9:16. [PubMed: 24885175]
108. Liu Y, et al. IKKbeta Deficiency in Myeloid Cells Ameliorates Alzheimer's Disease-Related Symptoms and Pathology. *J Neurosci*. 2014; 34:12982–12999. [PubMed: 25253847]
109. Durazzo TC, Mattsson N, Weiner MW. Smoking and increased Alzheimer's disease risk: a review of potential mechanisms. *Alzheimers Dement*. 2014; 10:S122–S145. [PubMed: 24924665]
110. Moreno-Gonzalez I, Estrada LD, Sanchez-Mejias E, Soto C. Smoking exacerbates amyloid pathology in a mouse model of Alzheimer's disease. *Nat Commun*. 2013; 4:1495. [PubMed: 23422663]
111. Ulrich JD, et al. Altered microglial response to Abeta plaques in APPS1–21 mice heterozygous for TREM2. *Mol Neurodegener*. 2014; 9:20. [PubMed: 24893973]
112. Cady J, et al. TREM2 variant p.R47H as a risk factor for sporadic amyotrophic lateral sclerosis. *JAMA Neurol*. 2014; 71:449–453. [PubMed: 24535663]

113. Rayaprolu S, et al. TREM2 in neurodegeneration: evidence for association of the p.R47H variant with frontotemporal dementia and Parkinson's disease. *Mol Neurodegener.* 2013; 8:19. [PubMed: 23800361]
114. Melnikova T, et al. Reversible pathologic and cognitive phenotypes in an inducible model of Alzheimer-amyloidosis. *J Neurosci.* 2013; 33:3765–3779. [PubMed: 23447589]
115. Kim J, et al. Normal cognition in transgenic BRI2-Abeta mice. *Mol Neurodegener.* 2013; 8:15. [PubMed: 23663320]
116. Berger-Sweeney J, et al. 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]
117. Gao Y, Pimplikar SW. The gamma -secretase-cleaved C-terminal fragment of amyloid precursor protein mediates signaling to the nucleus. *Proc Natl Acad Sci USA.* 2001; 98:14979–14984. [PubMed: 11742091]
118. Dodart JC, et al. Immunization reverses memory deficits without reducing brain Aβ burden in Alzheimer's disease model. *Nature Neurosci.* 2002; 5:452–457. [PubMed: 11941374]
119. Zheng H, Koo EH. Biology and pathophysiology of the amyloid precursor protein. *Mol Neurodegener.* 2011; 6:27. [PubMed: 21527012]
120. Bero AW, et al. Neuronal activity regulates the regional vulnerability to amyloid-beta deposition. *Nature Neurosci.* 2011; 14:750–756. [PubMed: 21532579]
121. Kang JE, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science.* 2009; 326:1005–1007. [PubMed: 19779148]
122. Xie L, et al. Sleep drives metabolite clearance from the adult brain. *Science.* 2013; 342:373–377. [PubMed: 24136970]
123. Siegel SJ, Bieschke J, Powers ET, Kelly JW. The oxidative stress metabolite 4-hydroxynonenal promotes Alzheimer protofibril formation. *Biochemistry.* 2007; 46:1503–1510. [PubMed: 17279615]
124. Head E, et al. Oxidation of Aβ and plaque biogenesis in Alzheimer's disease and Down syndrome. *Neurobiol Dis.* 2001; 8:792–806. [PubMed: 11592849]
125. Kress BT, et al. Impairment of paravascular clearance pathways in the aging brain. *Ann Neurol.* 2014; 76:845–861. [PubMed: 25204284]
126. Zhao W, Zhang J, Davis EG, Rebeck GW. Aging reduces glial uptake and promotes extracellular accumulation of Aβ from a lentiviral vector. *Front Aging Neurosci.* 2014; 6:210. [PubMed: 25177293]
127. Mawuenyega KG, et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science.* 2010; 330:1774. [PubMed: 21148344]
128. Hipp MS, Park SH, Hartl FU. Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol.* 2014; 24:506–514. [PubMed: 24946960]
129. Holmes C, et al. Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet.* 2008; 372:216–223. [PubMed: 18640458]
130. Boche D, et al. Reduction of aggregated Tau in neuronal processes but not in the cell bodies after Aβ42 immunisation in Alzheimer's disease. *Acta Neuropath.* 2010; 120:13–20. [PubMed: 20532897]
131. Serrano-Pozo A, et al. Beneficial effect of human anti-amyloid-beta active immunization on neurite morphology and tau pathology. *Brain.* 2010; 133:1312–1327. [PubMed: 20360050]
132. Vellas B, et al. Long-term follow-up of patients immunized with AN1792: reduced functional decline in antibody responders. *Curr Alzheimer Res.* 2009; 6:144–151. [PubMed: 19355849]
133. Mills SM, et al. Preclinical trials in autosomal dominant AD: implementation of the DIAN-TU trial. *Rev Neurol (Paris).* 2013; 169:737–743. [PubMed: 24016464]
134. Reiman EM, et al. Alzheimer's Prevention Initiative: a plan to accelerate the evaluation of presymptomatic treatments. *J Alzheimers Dis.* 2011; 26(Suppl 3):321–329. [PubMed: 21971471]

Figure 1.

An updated framework of the amyloid hypothesis. The black arrows illustrate the processing of APP by β - and γ -secretases to yield $A\beta$ species, which subsequently aggregate, ultimately triggering tau aggregation and downstream toxicity. Blue text and arrows illustrate proposed modifiers of the $A\beta$ cascade, while red text and arrows show the influence of aging and comorbid pathologies. Note that several feed-forward cycles are hypothesized, including one involving disturbed sleep promoting $A\beta$ production (and perhaps $A\beta$ clearance, though not depicted), while $A\beta$ aggregation in turn disrupts sleep cycles. Multiple factors, from aging to oxidative stress, contribute to proteostatic failure, which in turn promotes aggregation of $A\beta$, tau, and likely other toxic proteins. Many of the $A\beta$ modifying factors interact with each other (such as ApoE modulating inflammation) though this is not depicted.