# Neurobiology of Alzheimer's Disease: Integrated Molecular, Physiological, Anatomical, Biomarker, and Cognitive Dimensions

Joel Raskin[1,*], Jeffrey Cummings[2], John Hardy[3], Kory Schuh[1] and Robert A. Dean[1]

[1]*Eli Lilly and Company, Indianapolis, IN, USA;* [2]*Cleveland Clinic, Lou Ruvo Center for Brain Health, Las Vegas, NV, USA;* [3]*Institute of Neurology, University College London, UK*
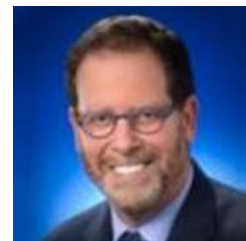
Joel Raskin

Abstract: ~~Background~~: Alzheimer's disease (AD), the most common form of dementia, is a progressive neurodegenerative disorder with interrelated molecular, physiological, anatomical, biomarker, and cognitive dimensions. ~~Methods~~: This article reviews the biological changes (genetic, molecular, and cellular) underlying AD and their correlation with the clinical syndrome. ~~Results~~: Dementia associated with AD is related to the aberrant production, processing, and clearance of beta-amyloid and tau. Beta-amyloid deposition in brain follows a distinct spatial progression starting in the basal neocortex, spreading throughout the hippocampus, and eventually spreading to the rest of the cortex. The spread of tau pathology through neural networks leads to a distinct and consistent spatial progression of neurofibrillary tangles, beginning in the transentorhinal and hippocampal region and spreading superolaterally to the primary areas of the neocortex. Synaptic dysfunction and cell death is shown by progressive loss of cerebral metabolic rate for glucose and progressive brain atrophy. Decreases in synapse number in the dentate gyrus of the hippocampus correlate with declining cognitive function. Amyloid changes are detectable in cerebrospinal fluid and with amyloid imaging up to 20 years prior to the onset of symptoms. Structural atrophy may be detectable via magnetic resonance imaging up to 10 years before clinical signs appear. ~~Conclusion~~: This review highlights the progression of biological changes underlying AD and their association with the clinical syndrome. Many changes occur before overt symptoms are evident and biomarkers provide a means to detect AD pathology even in patients without symptoms.

Keywords: Alzheimer's disease, anatomical, biomarker, cognition, molecular, neurobiology, physiological.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia, accounting for 60% to 80% of cases. Early symptoms typically include difficulty remembering names and recent events, apathy, and depression. Later symptoms include worsening memory, impaired judgment, disorientation, confusion, behavioral changes, and difficulty speaking, swallowing, and walking [1].

For clinical research, the classification of AD has recently been divided into 3 phases [2, 3]. First is a presymptomatic phase during which people are cognitively normal but with evidence of amyloid deposition with or without other neuropathological changes. Second is a symptomatic prodromal phase characterized by mild cognitive impairment (MCI) with amyloid deposition and more diverse evidence of neurodegeneration. In Individuals with MCI due to AD or prodromal AD experience a progressive cognitive decline greater than expected for their age and education level but without obvious signs of impaired function. The third phase occurs when cognitive impairment worsens and interferes with activities of daily living. The patient is then diagnosed

with dementia and has a full repertoire of molecular and neurodegenerative changes.

AD is a progressive disorder with interrelated molecular, physiological, anatomical, and clinical changes [4]. This review describes these domains and the progression of biological changes (genetic, molecular, and cellular) that underlie AD and their correlation with the clinical syndrome.

GENETICS OF AD

Established Genetic Risks for Sporadic or Late-Onset AD

The most common risk genes associated with AD susceptibility have roles in lipid processing, immune function, endocytosis, or synaptic integrity (Fig. **1**). Many genetic risk factors are associated with late-onset AD (after 65 years of age or older). Apolipoprotein E (APOE) is the most well-known risk factor gene. APOE is involved in cholesterol transport in CSF and in binding and clearance of beta-amyloid (Aβ) in the brain [5]. Of its 3 major alleles (ε2, ε3, and ε4), the APOE ε4 allele confers the greatest risk for developing late-onset familial and sporadic AD, most likely by reducing cholesterol efflux from neuronal cells and astrocytes, and by binding and depositing Aβ [5]. The prevalence of this allele is approximately 15% in the general population and approximately 40%in patients with AD [6]. The ε2 allele appears to play a protective role against AD [5].

*Address correspondence to this author at the Eli Lilly and Company, Indianapolis IN 46285, USA; Tel: 416-699-7260; Fax: 416-699-7352; E-mail: raskin_joel@lilly.com
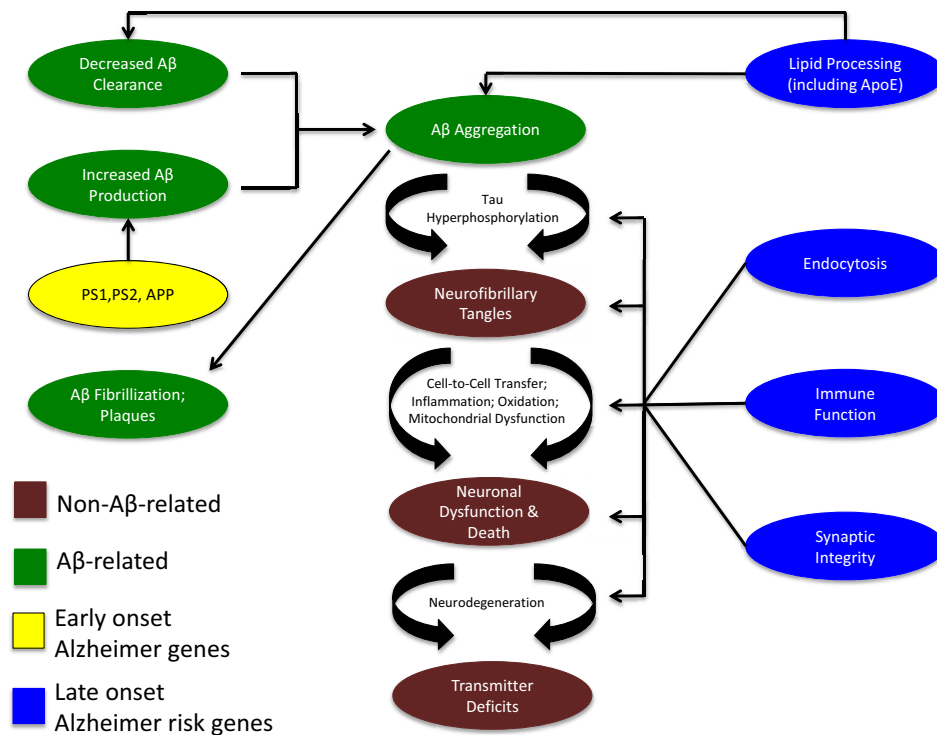
Fig. (1). Overview figure integrating genetic and pathologic aspects of Alzheimer's disease. Double bowed arrows imply multifaceted interacting processes. Colors indicate the major categories of activities involved. Dysregulated lipid processing can lead to Aβ aggregation and/or decreased clearance. Aβ aggregation is compounded by increased Aβ production related to early onset genes. Tau hyperphosphorylation can produce neurofibrillary tangles, cell death, and transmitter deficits. These processes are influenced by late onset genes affecting endocytosis, immune function, and synaptic integrity. Abbreviations: Aβ = β-amyloid; ApoE = apolipoprotein E; APP = amyloid precursor protein; PS1 = presenilin 1; PS2 = presenilin 2.

In addition to APOE, risk genes associated with lipid processing include ABCA7, clusterin (CLU), and sortilin-related receptor L (SORL1). ABCA7 encodes an adenosine triphosphate (ATP)-binding cassette transporter and plays multiple roles including substrate transport across cell membranes, regulation of amyloid precursor protein (APP) processing, and inhibition of Aβ secretion [7, 8]. CLU, a major brain apolipoprotein that reversibly and specifically binds Aβ and appears to act as a molecular chaperone, influences Aβ aggregation, deposition, conformation, and toxicity [9]. SORL1 is involved in vesicle trafficking from the cell surface to the Golgi-endoplasmic reticulum. It directs APP to endocytic pathways for recycling and plays an important role in Aβ generation [8].

Phosphatidylinositol-binding clathrin assembly protein (PICALM) gene and bridging integrator 1 are implicated in cell-cell communication and transduction of molecules across the membrane. CD33 is a member of the sialic-acid-binding immunoglobulin-like lectins (Siglec) family which is thought to promote cell-cell interactions and regulate functions of cells in the innate and adaptive immune systems [7]. The gene TREM2 has a role in modulating risk for late-onset AD [10] and heterozygous rare variants are associated with a significant increase in the risk of AD [11]. TREM2 is an innate immune receptor expressed on the cell surface of microglia, macrophages, osteoclasts, and immature dendritic cells; it triggers the activation of immune responses. CD2-associated protein is a scaffold/adaptor protein that associ-

ates with proteins involved in receptor-mediated endocytosis. EPHA1 is a member of the ephrin receptor subfamily and a membrane-bound protein that plays a role in cell and axon guidance, cell morphology and motility, and apoptosis and inflammation. Membrane-spanning 4A gene cluster, which encodes the beta subunit of high-affinity IgE receptors and complement receptor 1 (CR1), also plays a role in immune response [7].

MicroRNAs (miRNAs) are small non-coding, single stranded RNAs that currently represent the smallest known carriers of highly selective genetic regulatory information in the human CNS [12]. Recent findings suggest that miRNAs are directly related to AD. Dysregulation of miRNA profile is being investigated as a mechanism for AD pathogenesis by interrupting the metabolism of amino acids in the brain [13]. miR-153 has been shown to inhibit expression of APP in human neurons; low miR-153 levels may drive increased APP expression in a subset of AD patients [14]. Additionally, miR-339-5p has been shown to negatively regulate BACE1 and Aβ in human brain cultures and is dysregulated in the AD brain [15]. Serum circulating miRNAs are being investigated as peripheral biomarkers of AD [16]. The pro-inflammatory miRNA-34a demonstrates a significant up-regulation in AD leading to speculation that an anti-miRNA-34a may be useful in the future clinical management of AD [12]. These and other recent miRNA findings suggest new venues for identifying novel targets for slowing AD progression [17].

Establishing Genetic Causes of Early-Onset AD

Dominant familial genes related to Aβ processing are genetic causes of early-onset AD. Presenilin 1 (PSEN1) and presenilin 2 (PSEN2) are components of γ-secretase. This multiprotein complex required for Aβ production consists of 4 essential subunits: PSEN; nicastrin; anterior pharynx defective; and presenilin enhancer 2 (PEN2), which assemble in a 1:1:1:1 stoichiometry [18]. Mutations can alter the production of Aβ42. The APP gene encodes the APP peptide. Mutations in this gene result in preferential processing through the amyloidogenic pathway [19]. These genes account for a small percentage of AD cases and typically occur in patients with the onset of clinical symptoms midlife [19, 20].

NEUROPATHOLOGICAL BASIS FOR CLINICAL SYMPTOMS

There are 3 pathognomonic types of pathological changes detectable in the brain – beta amyloid (Aβ)-containing plaques, tau-containing neurofibrillary tangles, and atrophy. Each pathological element has corresponding associated biomarkers:

1. Aβ plaque–The majority of patients with a correct clinical diagnosis of AD have extra-neuronal neuritic plaques evident by post-mortem histopathological examination, antemortem amyloid positron emission tomography (PET) imaging, or low concentrations of cerebral spinal fluid (CSF) Aβ_{1-42}.

2. Neurofibrillary tangles - Tangles are intraneuronal fibrils primarily composed of abnormal, hyperphosphorylated forms of the microtubule-associated protein tau. Increased CSF tau is not specific for AD but does correlate with clinical disease severity. Phosphorylated tau (p-tau) is the protein of neurofibrillary tangles and is a more AD-specific protein than is total tau (t-tau).

3. Brain atrophy - The rate and distribution of brain atrophy can be predictive of cognitive impairment and dementia [21]. Specific patterns of brain atrophy as measured by volumetric magnetic resonance imaging (vMRI) may be a potential biomarker for AD; vMRI shows a strong correlation between brain atrophy and severity of cognitive impairment [22].

Other neuropathological changes include synaptic loss, neuronal loss, gliosis, degenerative changes in white matter, granulovacuolar degeneration, cerebral amyloid angiopathy, and other protein aggregates [23].

BETA-AMYLOID PLAQUES

Amyloid Cascade Hypothesis

The amyloid cascade hypothesis (Fig. **2**) proposes that AD is caused by an imbalance between Aβ production and clearance, resulting in increased amounts of Aβ monomers, oligomers, insoluble fibrils, and plaques [24, 25]. During normal Aβ trafficking, APP is produced in large quantities in the neurons and metabolized rapidly through both non-amyloidogenic and amyloidogenic pathways [26]. From the trans-Golgi network (TGN), APP can be transported either to the plasma membrane or directly to an endosomal compart-

ment [26, 27]. APP is first cleaved within the luminal domain by β- or α-secretase. APP can be metabolized by α-secretase and then γ-secretase at the plasma membrane, leading to the release of secreted APP (sAPP) α and a peptide (p3) into the extracellular space [26]. APP can be metabolized by β-secretase BACE1 and γ-secretase in the endosomes, leading to release of Aβ into the extracellular space or the degradation of Aβ in lysozymes. Additionally, a fraction of the APP released into the extracellular space may be rapidly reinternalized via clathrin-coated pits into endosomes and metabolized in the Golgi, TGN, or endosomes by BACE1 and γ-secretase into Aβ [26, 27].

Aβ is cleared from the central nervous system through a number of mechanisms, including vascular mechanisms, phagocytosis from microglia, or enzymatic breakdown [28]. Along with clearance through arterial pathways, Aβ can be cleared through perivascular drainage of interstitial fluid through the basement membranes of capillaries in the brain [29]. Capillary and artery basement membranes drain fluid and solutes from the brain. Aβ is deposited in these drainage pathways in cerebral amyloid angiopathy and may impede elimination of Aβ and interstitial fluid from the brain in Alzheimer's disease [30]. Gradual changes in the steady-state levels of Aβ in the brain are thought to initiate a cascade of pathological events in AD that lead to neuronal dysfunction, cell death, and dementia [25, 31]. Aβ levels can be elevated by enhanced production and/or reduced clearance [31]. PSEN1 and PSEN2 are associated with increased production of Aβ in dominantly inherited AD, whereas late-onset AD is associated with decreased Aβ clearance. Most late-onset AD is due to reduced clearance and most early-onset AD is due to over production, with a few homozygotes e4 slow clearers.

Aβ40 and Aβ42/43 are the main toxic isoforms; Aβ42 is more hydrophobic, toxic, and prone to form oligomers, fibrils, and amyloid plaque than Aβ40 and other shorter Aβ fragments [32]. Kinetic studies with labeled Aβ42 and Aβ40 in patients with AD showed that Aβ production rates did not differ between cognitively normal control and AD groups, but the average clearance rate of Aβ was slower for patients with AD than for controls. Late-onset AD is associated with a 30% impairment in clearance of both Aβ42 and Aβ40 [33]. These results require confirmation but support the proposal of decreased Aβ clearance in late-onset AD.

The segregation of APP and β-secretase into cholesterol-rich lipid rafts in the early Golgi, late Golgi/early endosomes, and endosomes may be a crucial element in the amyloidogenic pathway [26]. Although APP inside raft clusters seems to be cleaved by β-secretase, APP outside rafts undergoes cleavage by α-secretase. Therefore, Aβ generation due to access of α- and β-secretase to APP may be determined by dynamic interactions of APP with lipid rafts [34].

Results from recently completed trials can provide insight into the viability of the amyloid cascade hypothesis. Studies of a γ-secretase inhibitor found that patients experienced cognitive decline in the placebo group and the 2 γ-secretase groups with patients in the higher dose group experiencing significant worsening of functional ability [35]. The γ-secretase reduced plasma levels of Aβ but not CSF levels. This suggests that the target was engaged peripherally rather
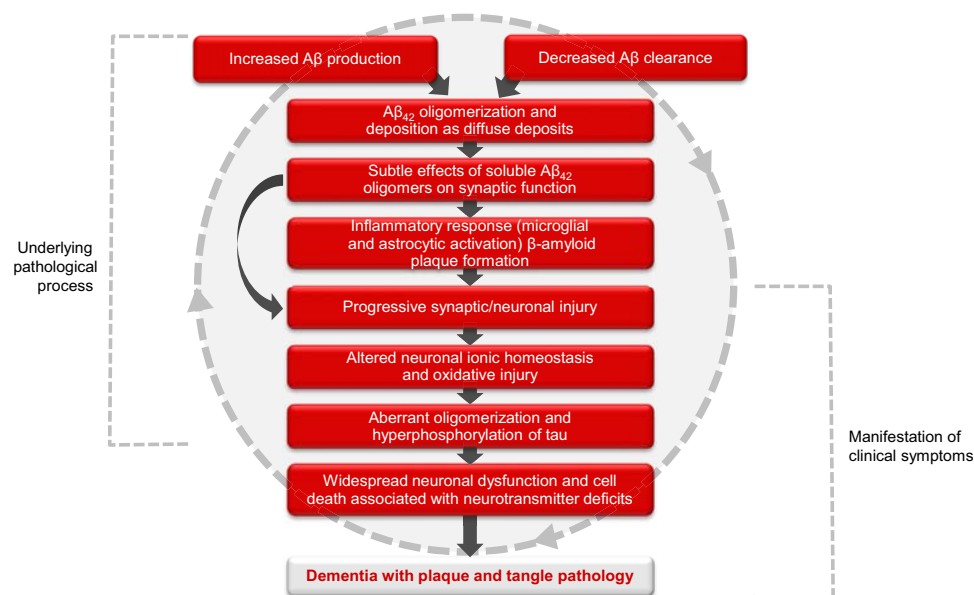
Fig. (2). Amyloid cascade hypothesis [25,31] as a model of the pathological events in AD that are triggered by changes in Aβ metabolism. (Copyright 2014 Eli Lilly and Company). Gradual changes in the steady-state levels of Aβ in the brain are thought to initiate a cascade of pathological events in AD that leads to neuronal dysfunction, cell death, and dementia. The strict linearity implied by this model does not illustrate the true complexity of the interactions. Changes in Aβ metabolism, in particular the $A\beta_{42}:A\beta_{40}$ ratio, may result from missense mutations in APP, PSEN1, or PSEN2. The relative increase in $A\beta_{42}$ enhances oligomer formation, which causes subtle and increasingly severe changes in synaptic function. As Aβ forms plaques, local inflammatory glial responses are observed in microglial and astrocytic cells. Synaptic spine loss and neuritic dystrophy occur. Over time, these events result in oxidative stress and altered neuronal ionic homeostasis, as well as other biochemical changes. Tau protein is hyperphosphorylated, leading to aberrant oligomerization and the development of neurofibrillary tangles. The cascade culminates in widespread synaptic and neuronal dysfunction and cell death. Eventually, the cascade leads to progressive dementia associated with extensive Aβ and tau pathology. Abbreviations: Aβ = β-amyloid.

than in the brain [35]. A subsequent essay by De Strooper (2014) [36] reported that this negative trial severely dampened enthusiasm for γ-secretase research but argued that research should continue that incorporates the knowledge gained from the trial.

There is strong evidence for the idea that reducing BACE1 cleavage of APP might protect against AD. Evidence was provided by researchers who examined the coding variants in APP in a set of whole-genome sequence data from 1795 Icelanders. These researchers found a coding mutation in the APP gene that protects against AD [37]. Reducing BACE1 cleavage of APP might protect against AD with the therapeutic potential to reduce amyloid burden and block amyloid toxic effects [38].

A monoclonal antibody that binds to soluble Aβ, showed an increase in Aβ in the CSF and plasma, along with a potential cognitive effect in patients with mild AD. There was no effect in patients with moderate AD [39]. This supports the amyloid cascade hypothesis in that earlier treatment may be beneficial when using amyloid-targeting agents.

Progression of Aβ

Aβ deposits in the brain follow a distinct spatial progression starting in the basal neocortex, spreading throughout the hippocampus**,** and eventually spreading throughout the rest of the cortex [40]. In the first stage (Braak Stage A), Aβ plaques are initially found in the basal neocortex, most frequently in the perirhinal and/or entorhinal fields. In the sec-

ond stage (Braak Stage B), Aβ deposition increases and spreads into adjoining neocortical areas and the hippocampus. Eventually, in the third stage (Braak Stage C), deposits are found in all areas of the cortex, including the densely myelinated primary areas of the neocortex. Aβ is not commonly detected in temporal medial structures in PET scans perhaps due to a resolution/partial volume effect and because it is not an early site for Aβ as it is for tau tangles.

Cell Death via Intracellular Accumulation and Nucleation of Aβ

Studies using a cell culture system that reproducibly leads to the formation of Aβ amyloid plaques demonstrated that the formation of a single plaque represents a template-dependent process dependent upon the presence of endocytotic- or phagocytotic-competent cells. Induced plaque formation in a variety of monocytic cells suggests that soluble extracellular Aβ peptides become internalized and sorted in intracellular vesicles, such as multivesicular bodies (MVBs). Aβ peptides may nucleate within the MVBs and form fibrils that grow and penetrate the vesicular membrane, ultimately causing cell death and releasing Aβ into the extracellular space [41].

Soluble and Insoluble Forms of Aβ

Aβ exists in multiple forms including soluble monomeric, soluble oligomeric, and insoluble amyloid fibrils and plaques [42]. Many kinds of oligomers have been reported

and they often exist in rapid equilibrium with monomers and higher-order assemblies [43]. The proteolysis of APP into soluble Aβ monomers results in peptides of 39-42/43 residues in length [44]. Soluble random coil Aβ monomers may misfold into beta sheets and self-associate into soluble dimers, trimers, and oligomers, eventually giving rise to insoluble fibrils and plaques [45]. Soluble Aβ oligomers appear to be neurotoxic, inducing synaptic dysfunction [31]. The inert appearance of insoluble amyloid plaques has led to the suggestion that their formation may be a neuroprotective role. Amyloid plaques may serve as a reservoir for the smaller, soluble, and potentially neurotoxic oligomeric Aβ42 [46]. However, the neurotoxicity of Aβ could be an indirect effect via microglia activation as described below.

Aβ Outside Normal Physiological Levels Impairs Synaptic Activity

Aβ may be a positive regulator of synaptic transmission. Aβ release into the extracellular space is part of a feedback loop controlling neuronal excitability; small increases of Aβ within a physiological range facilitate presynaptic function. Intermediate levels of Aβ (including Aβ42) may enhance presynaptic activity whereas abnormally low levels of Aβ reduce presynaptic efficiency and abnormally high levels of Aβ depress postsynaptic transmission and lead to a loss of dendritic spines [47].

Elevated Glial Cell Inflammatory Response to Aβ

Genetic, cellular, and molecular changes associated with AD point to the involvement of immune and inflammatory processes. However, it is not yet known whether inflammation is a cause, contributor, or secondary phenomenon in AD [48]. Microglia, the brain's resident macrophages, are pivotal players in immune/inflammatory response in multiple neurological disorders, including AD. They remove redundant, apoptotic neurons. They secrete a wide variety of inflammatory factors, including reactive oxygen species, T cell-1 cytokines, chemokines, growth factors, and complement components. These inflammatory factors are elevated in pathologically vulnerable regions of the AD brain. The dense accumulation of microglia within and around Aβ deposits suggests that aggregated Aβ deposits are potent stimuli for inflammatory responses [48]. It is hypothesized that amyloid deposition is the primary event in Alzheimer pathogenesis. While this deposition is being dealt with by microglia in their phagocytic mode, there are few consequences (high amyloid controls). When the microglia become overwhelmed, they switch to inflammatory (cytokine production) mode, then neuronal toxicity and neurodegeneration are initiated [49]. A recent study investigated the relationship between AD, inflammation, and dyslipidemia using summary statistics from genome-wide association studies of over 200,000 individuals [50]. The investigators found genetic overlap between AD, C-reactive protein, and plasma lipids, and identified novel AD susceptibility loci including 2 genome-wide significant variants associated with increased risk for AD. The potential involvement of immune and inflammatory processes suggests that anti-inflammatory medications (e.g., nonsteroidal anti-inflammatory drugs) might be beneficial. However, trials have produced mixed results [48].

Astrocytes play an essential neurosupportive role in the brain, including secretion and recycling of neurotransmitters, ion homeostasis, energy metabolism regulation, synaptic remodeling, and modulation of oxidative stress. Reactive astrocytes encircle Aβ deposits similar to glial scarring, a mechanism by which the cells may provide a barrier between healthy tissue and areas of injury or infection. Astrocytes may mediate aspects of AD neuropathology through expression or overexpression of inflammation-related factors. Secreted reactive species include cytokines, S100β (neurotropin that induces neurite proliferation), interleukin 1β, tumor necrosis factor-α, inducible nitric oxide synthase, and nitric oxide [48, 51].

Oligodendrocytes are critical for neurotransmission. They produce a myelin sheath that envelops axons and speeds neuronal transmission. Studies have revealed myelin abnormalities in AD white matter and focal demyelination of axons in proximity to Aβ deposits in gray matter. Oligodendrocytes have been reported to be immunoreactive for complement components C1q, C1s, C2-C9 [48].

Model of Synaptic Dysfunction caused by Aβ-Associated Mitochondrial Dysfunction

Oxidative stress is one of the earliest neuropathological events of AD [52]. It is a consequence of a marked imbalance between tissue production and subsequent removal of reactive oxygen species by the antioxidant system. A 2011 report purports that oxidative stress occurs early in the progression of AD pathology, before detection of established histological hallmarks of AD [52]. Oxidative stress and free radicals (O_2^*, H_2O_2, OH) activate β-secretase and facilitate cleavage of APP to Aβ [53]. Aβ may enter mitochondria, further induce free radicals, decrease cytochrome oxidase activity, decrease ATP production, decrease anterograde mitochondrial trafficking, increase mitochondrial fragmentation, increase hyperphosphorylation of tau, and eventually induce synaptic dysfunction [52-54].

Stöhr *et al.* (2012) [55] reported that Aβ aggregates are prions and that Aβ alone is sufficient for the formation of a self-propagating protein assembly. A recent report by Walker *et al.* (2013) [56] suggests that the spread of lesions from one site to another is mediated by the cellular uptake, transport, and release of endogenous seeds formed by the cognate proteins.

NEUROFIBRILLARY TANGLES

Formation of Neurofibrillary Tangles

Kinases and phosphatases maintain physiologic levels of phosphorylated and nonphosphorylated forms of tau. Hyperphosphorylated tau is found in 2 subcellular pools in AD brains: neurofibrillary tangles of paired helical filaments mixed with twisted ribbons and straight filaments and nonfibrillized tau in the cytosol. Levels of tau protein are increased 4- to 8-fold in AD brains as compared to control brains [57].

Hyperphosphorylated tau lacks affinity for microtubules, and also sequesters normal tau, microtubule-associated protein (MAP) 1, and MAP2, causing the inhibition and disassembly of microtubules [57]. As the phosphorylation of tau

increases, tau becomes insoluble and self-assembles into wide, loosely coiled paired helical filaments and more tightly coiled straight filaments, which form the neurofibrillary tangles [57, 58]. Upon aggregation into filaments, tau loses the ability to sequester normal tau, indicating that, similar to plaque, the formation of filaments may be a self-defense response initiated by the affected neurons [57].

The concentrations of both p-tau and t-tau are increased in CSF of individuals subsequently shown to have autopsy-proven AD [59]. While CSF tau is not specific for AD, levels do correlate with clinical disease severity. CSF p-tau purportedly possesses greater diagnostic specificity than t-tau for AD and has been used to differentiate AD from a variety of non-AD degenerative neuropathies [60].

Progression of Tau

Transgenic mouse models suggest that AD-related neuronal dysfunction spreads through anatomically connected networks, between connected and vulnerable neurons. In a study by Liu *et al.*, (2012) [61], transgenic mice express human tau in the entorhinal cortex. The hippocampus was immunostained with antibody against abnormal tau, and in young mice (10 to 11 months) abnormal human tau was most abundant in the medial entorhinal cortex, lateral entorhinal cortex, and parasubiculum. In older mice (22 months), tau was distributed to the superficial layers of the entorhinal cortex, throughout the subiculum, in CA1, and in dentate gyrus granule cells. The pattern of tau localization supports a trans-synaptic mechanism of spread along anatomically connected networks, between connected and vulnerable neurons.

Newly developed PET tracers that bind with tau tangles are being developed. Based on studies of samples obtained at autopsy, the amount and location of tau tangles in an AD patient's brain is thought to correlate with the severity of the disease [62].

Hyperphosphorylated Tau Disruption of Axonal Transport

The cellular functions of normal tau include stabilization of microtubules, promotion of neurite outgrowth, membrane interactions, facilitation of enzyme anchoring, and facilitation of axonal transport of organelles to nerve terminals [52]. Mandelkow *et al.* (2003) [63] found that tau is capable of reducing net anterograde transport of vesicles and cell organelles to the synapse by blocking the microtubule tracks. Hyperphosphorylated tau adopts an altered conformation and relocalizes from axonal to somatodendritic compartments [64]. The long-distance intracellular transport of vesicles and organelles along the axon to maintain nutritional supply and clear away waste products is mainly achieved by microtubule-dependent axonal transport [64, 65]. Microtubules serve as rail tracks, and motor proteins, such as kinesins, serve as engines transporting organelles from the cell body to the nerve terminals [52]. Hyperphosphorylation of tau depolymerizes microtubules, which impairs anterograde transport of organelles to the synapse, including mitochondria, and in turn leads to neurodegeneration [64, 65].

Tau and Aβ Pathology Both Occur in Regions of the Brain Associated with Cognition

Aβ and p-tau are co-localized in AD synaptosomes. Fein *et al.* (2008) [66] used flow cytometry analysis of synaptosomes in fresh AD postmortem tissue to demonstrate that AD synaptic terminals contain both Aβ and p-tau. An average of 68.4% of synaptic terminals was positive for Aβ and 32.3% positive for p-tau. Synaptic p-tau was highest in the entorhinal cortex and hippocampus. Synaptic Aβ fluorescence was lower in the entorhinal cortex and hippocampus relative to neocortical regions. Synaptic Aβ and p-tau fluorescence was significantly correlated and dual-labeling experiments demonstrated that 24.1% of Aβ-positive terminals were also positive for p-tau. The highest fraction of dual labeling (39.3%) was in the entorhinal cortex, the earliest affected region.

Glucose Metabolism

Progressive Decrease in Neural Glucose Metabolism

AD is marked by synaptic dysfunction and cell death accompanied by a corresponding progressive loss of cerebral metabolic rate for glucose (CMRglc) [67]. CMRglc reduction is first detected in the hippocampus in early-stage AD and progresses to the parietotemporal and posterior cingulate cortex [67]. CMRglc reduction can precede the onset of clinical symptoms by several years and correlates with dementia severity and pathological diagnosis of AD [67, 68]. Fluorodeoxyglucose [^{18}F] positron emission tomography (FDG-PET) measures glucose uptake in the brain in the mental resting state; this reflects cerebral metabolic activity [69] and integrated synaptic activity [70-72].

FDG-PET, an indicator of synaptic activity, may potentially serve as a marker for AD [22]. Decreased FDG-PET uptake is an indicator of impaired synaptic function in patients with AD. FDG-PET studies in patients with AD show a specific topographic pattern of decreased glucose uptake in a lateral temporal-parietal and posterior cingulate, precuneus distribution. Decreased PET activity may reflect loss of neurons or abnormal function of remaining neurons based on insulin resistance and glucose utilization [73].

Dysfunctional Insulin Signaling

It has been proposed that dysfunctional insulin signaling in the brain could be involved with AD [74, 75]. Insulin and insulin receptors are distributed throughout the brain and are impaired in the brains of patients with AD [76]. It has been proposed that defects in the brain insulin signal transduction system and the associated consequences are involved in the pathogenesis of AD [76]. This area of research could yield novel methods of treatment; trials are ongoing.

CELL LOSS AND STRUCTURAL DYSTROPHY

Progressive Synaptic Loss Associated with AD and its Correlation to Cognitive Decline

Synaptic loss in the hippocampus and neocortex is an early event and the major structural correlate of cognitive dysfunction [77]. Quantitative ultrastructural studies on the temporal and frontal cortical biopsies 2 to 4 years after onset of clinical disease reveal a decrease in the density of both numerical synapses and number of synapses per neuron [77]. Synapse number was correlated with cognitive function by examining brain tissue from 28 individuals involved in 2

separate longitudinal studies. Individuals included in these studies agreed to annual clinical evaluations and to brain donation at time of death [78]. When the number of synapses of the hippocampal CA1 region was analyzed for subjects with no cognitive impairment (NCI), MCI, or mild AD (mAD), there was a significant decrease (p<.001) in the mAD group compared to both the MCI and NCI groups [79] (Fig. **3**).

Synaptic Damage may Contribute to Cognitive Decline in Aging Persons and Persons with AD

The hippocampal dentate gyrus outer molecular level receives a direct excitatory input from the ipsilateral entorhinal cortex, an area known to be affected early in the course of AD [78]. In a 2007 study, individuals with mAD had 55% fewer synapses than individuals with NCI and 45% fewer than individuals with MCI; individuals with MCI had 18% fewer synapses than those with NCI [79].

Severity of synaptic marker loss and regional differences within the cerebral cortex appear to reflect the pattern of neurofibrillary degeneration as outlined by Braak staging [77].

Progressive Decrease of Choline Acetyltransferase and Acetylcholinesterase Activity in Patients with AD and its Correlation to Cognitive Decline

There is a close relation between changes in the cholinergic system and AD dementia. Choline acetyltransferase (CAT) and acetylcholinesterase (AChE) activity progressively decreases in patients with AD [80]. The "cholinergic hypothesis" postulates functional abnormalities are associated with defective cholinergic neurotransmission. Cholinergic deficits of reduced CAT and AChE activity levels in postmortem brain samples and CSF are reported as the most severe and consistent with neurochemical changes in AD [81]. Patients with moderately severe AD were found to have an average of 25% to 33% decrease in mean cortical AChE activity [81]. With PET studies of the cerebral cortex of patients with mild to moderate AD, Kuhl *et al.* (1999) [82] found AChE activities were reduced by about 30%, and concomitantly measured cholinergic terminal densities were reduced by about 40% compared with normal controls. Decrease in CAT and AChE activity significantly correlates with Aβ plaque count and intellectual impairment, and it is associated with attention and working memory [80]. Additionally, AChE activity in plasma is correlated with brain Aβ load [83].

Patterns of Progression of Structural Atrophy of the Brain in Patients with MCI and Patients with AD

Anatomical signs of AD include progressive brain atrophy, particularly in the hippocampal region. Hippocampal baseline measures and atrophy rates best discriminate MCI from cognitively normal patients, suggesting hippocampal atrophy takes place early in AD relative to atrophy in other brain regions [84]. Whole-brain volume best discriminates AD from MCI, suggesting that whole-brain atrophy is readily evident in later-stage AD [84]. A meta-analysis showed an overall mean hippocampal atrophy rate of 4.7% per year (compared to 1.4% per year for matched control) [85].

Structural Atrophy may be Detectable via MRI up to 10 Years Before Clinical Signs Appear in Patients with AD

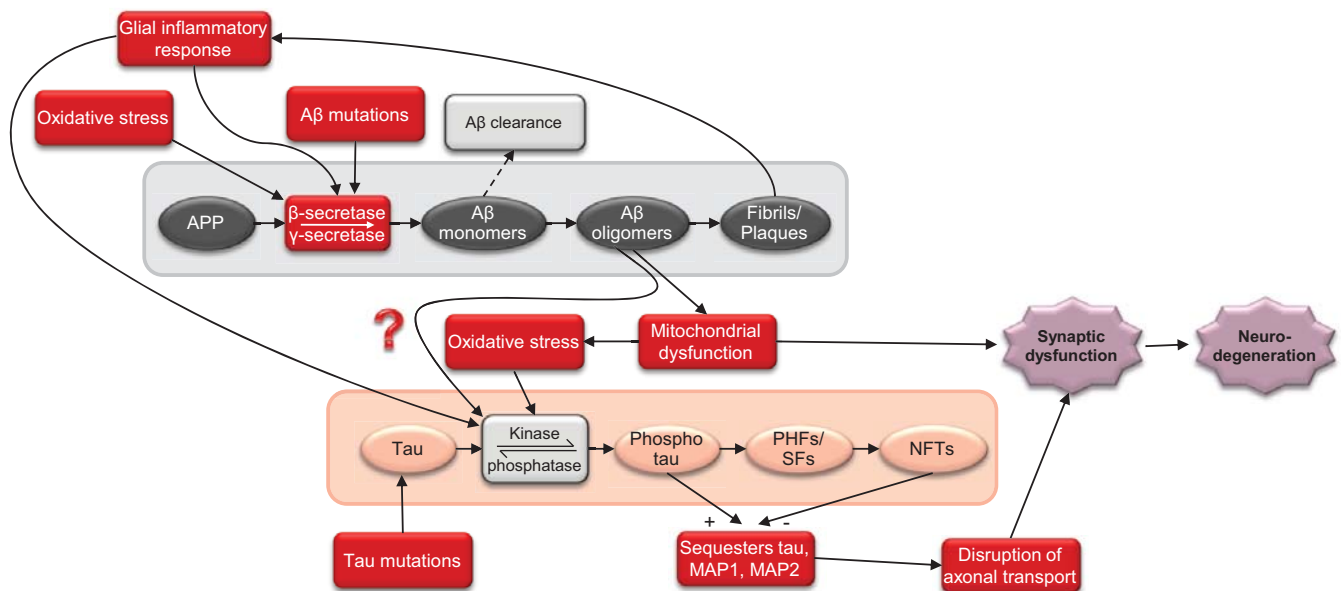Structural atrophy may be detectable via MRI in the preclinical asymptomatic phase of AD [86]. A group of 148

Fig. (3). Alzheimer's disease progression highlighting the molecular, physiological, anatomical, and cognitive changes. (Copyright 2014 Eli Lilly and Company). Dementia associated with AD is related to the aberrant processing and clearance of Aβ and tau. Feedback mechanisms associated with inflammatory responses and oxidative stress set in motion a cascade of pathological events. Cellular-level events lead to synaptic dysfunction and neurodegeneration in the brain. Abbreviations: AD = Alzheimer's disease; Aβ = β-amyloid; APP = amyloid precursor protein; PHFs = paired helical filaments; SFs = straight filaments; NFTs = neurofibrillary tangles; MAP = microtubule-associated protein.

cognitively normal volunteers were recruited from a longitudinal study [87]. Individuals were evaluated clinically and via MRI annually for 5 years and then re-evaluated 7 and 10 years after baseline. At each follow-up, clinical diagnoses of amnestic MCI and probable AD were made according to published criteria. A voxel-based morphometry study of gray matter density in preclinical patients with AD demonstrated that structural MRI changes may be detectable up to 10 years before clinical symptoms of AD occur [87].

An MRI study of cortical thinning in patients (older controls, incipient AD, very mild AD, and mAD) investigated both the entire cortical mantle and specific regions of interest in the brain. Several regions were 10% to 20% thinner than normal. The regions with the most thinning were those typically thought to be affected earliest in AD progression: medial temporal, inferior temporal, temporal pole, inferior parietal, and posterior cingulate/precuneus. The severity of the loss of cognitive symptoms (memory, orientation, judgment, and problem solving) was strongly associated with cortical thickness [88].

Diffusion tensor imaging (DTI) has been used to focus on the damage to white matter in the brain. Studies have found DTI indicators of impaired white matter in AD and MCI. Increased translational diffusion (mean diffusivity: MD) and decreased directional diffusion (fractional anisotropy: FA) damage to white matter has been assessed in AD. Regions of increased MD and decreased FA in patients with AD and MCI have been found in all lobes of the brain, as well as medial temporal lobe structures including the hippocampus, entorhinal cortex and parahippocampal white matter [89].

Evidence Showing that Synaptic Dysfunction Spreads through Connected Neural Networks

Transgenic mouse models suggest that AD-related dysfunction is propagated through synaptically connected neural networks, with the entorhinal cortex as an important hub region of early vulnerability [90]. The highest levels of Aβ were initially revealed in the entorhinal cortex at 6 months, but spread to anatomically connected regions by 13 months. The image of the hippocampus indicates connections from the entorhinal cortex to the dentate gyrus to the CA1 region, as well as reciprocal pathways from the entorhinal cortex to the retrosplenial cortex.

The Structure and Function of Default-Mode Network in AD

The resting brain shows ongoing metabolic activity known as the default network including the posterior cingulate cortex, adjacent precuneus, and the hippocampus [91, 92]. Default-mode network regions normally are active during episodic and autobiographical memory retrieval and envisioning the future. Default-mode network activity decreases during memory formation and goal-directed tasks.

Patients with AD show deficient default-mode network activity as compared to healthy elderly controls, particularly in the posterior cingulate and hippocampus [93]. Patients also show altered functional connectivity between brain regions [94]. Network analysis of intrinsic functional brain connectivity has shown that cognitive decline in AD is asso-

ciated with disrupted global functional organization in the brain [95]. Connections that are disrupted include the posterior cingulated cortex which is strongly connected with most of the default-mode network regions but tends to be attenuated in patients with AD [96].

The pattern of Aβ deposition in patients with AD as detected with Pittsburgh compound-B-PET shows substantial spatial overlap with the default-mode network. Greatest areas of overlap between Aβ and the default-mode network are in the precuneus/posterior cingulate, medial prefrontal, and angular gyri [97].

Time Sequence of Biomarkers

The change in biomarkers appears to have the following sequence [3]:

Sequence Order	Biomarker
1	An increase in CSF tau (although perhaps not reaching detection threshold until later)
2	A decrease in CSF Aβ as it accumulates in the brain
3	An increase in amyloid deposition using PET
4	Brain atrophy measured with MRI fMRI, and FDG PET
5	Cognitive impairment
6	Decreased clinical functioning and activities of daily living

CONCLUSION

Tying Together the Molecular, Physiological, Anatomical, and Cognitive Changes

The time course and levels of AD pathology highlight the connections between the molecular, physiological, anatomical, and cognitive changes. Dementia associated with AD is related to the aberrant processing and clearance of Aβ and tau. Feedback mechanisms associated with inflammatory responses and oxidative stress set in motion a cascade of pathological events. Cellular-level events lead to synaptic dysfunction and neurodegeneration in the brain. AD pathology begins in the hippocampus and spreads through neural networks associated with memory. In particular, AD appears to follow the default-mode network, associated with resting state episodic memory. Biomarkers for Aβ plaque, neurofibrillary tangles, and brain atrophy serve as the limited window on biology available to the clinician.

LIST OF ABBREVIATIONS

Aβ	=	Beta-amyloid
AChE	=	Acetylcholinesterase
AD	=	Alzheimer's disease
APOE	=	Apolipoprotein E
APP	=	Amyloid precursor protein

ATP	=	Adenosine triphosphate
CAT	=	Choline acetyltransferase
CLU	=	Clusterin
CMRglc	=	Cerebral metabolic rate for glucose
CSF	=	Cerebral spinal fluid
DTI	=	Diffusion tensor imaging
FA	=	Fractional anisotropy
FDG-PET	=	Fluorodeoxyglucose positron emission tomography
fMRI	=	Functional magnetic resonance imaging
mAD	=	Mild Alzheimer's disease
MAP	=	Microtubule-associated protein
MCI	=	Mild cognitive impairment
MD	=	Mean diffusivity
MRI	=	Magnetic resonance imaging
MVB	=	Multivesicular bodies
NCI	=	No cognitive impairment
PEN	=	Presenilin enhancer
PET	=	Positron emission tomography
PSEN	=	Presenilin
TGN	=	Trans-Golgi network
vMRI	=	Volumetric magnetic resonance imaging

CONFLICT OF INTEREST

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