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Molecular and Cellular Pathophysiology of Preclinical Alzheimer's Disease

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Abstract

Although the two pathological hallmarks of Alzheimer's disease (AD), senile plaques composed of amyloid- β (A β) peptides and neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau, have been studied extensively in postmortem AD and relevant animal and cellular models, the pathogenesis of AD remains unknown, particularly in the early stages of the disease where therapies presumably would be most effective. We and others have demonstrated that A β plaques and NFTs are present in varying degrees before the onset and throughout the progression of dementia. In this regard, aged people with no cognitive impairment (NCI), mild cognitive impairment (MCI, a presumed prodromal AD transitional state), and AD all present at autopsy with varying levels of pathological hallmarks. Cognitive decline, a requisite for the clinical diagnosis of dementia associated with AD, generally correlates better with NFTs than A β plaques. However, correlations are even higher between cognitive decline and synaptic loss. In this review, we illustrate relevant clinical pathological research in preclinical AD and throughout the progression of dementia in several areas including A β and tau pathobiology, single population expression profiling of vulnerable hippocampal and basal forebrain neurons, neuron plasticity, neuroimaging, cerebrospinal fluid (CSF) biomarker studies and their correlation with antemortem cognitive endpoints. In each of these areas, we provide evidence for the importance of studying the pathological hallmarks of AD not in isolation, but rather in conjunction with other molecular,

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cellular, and imaging markers to provide a more systematic and comprehensive assessment of the multiple changes that occur during the transition from NCI to MCI to frank AD.

Introduction

At the turn of the twentieth century, several prominent neurobiologists described the presence of extracellular lesions in the brain of people with dementia. Blocq and Marinesco (1892) reported abnormal argyrophilic extracellular plaques in post mortem brain tissue from two aged persons with dementia [1, 2], a finding confirmed by Emil Reich and Oskar Fisher (Fig. 1A) [3]. The German psychiatrist, Alois Alzheimer (Fig. 1B), was treating a 51 year old woman named Auguste Deter (Fig. 1C), who presented with signs of paranoia and memory loss, who subsequently died five years later. At autopsy, Alzheimer observed brain atrophy, neuronal loss, and dense argyrophilic fibrillary bundles in form of tangles (Fig. 1D, E) and plaques (Fig. 1F). In 1910, Dr. Emil Kraepelin, suggested that this triad of signs and symptoms be termed “Alzheimer's disease”. Today, senile plaques and neurofibrillary tangles (NFTs) are considered the defining pathological lesions of Alzheimer's disease (AD).

Amyloid plaques accumulate in the extracellular matrix (Fig. 1F) and consist of insoluble fibrils of amyloid-beta peptides ($A\beta$), which are cleaved from the larger transmembrane $A\beta$ precursor protein (APP) by successive cleavage through the beta-site APP cleaving enzyme 1 (BACE1) and the intramembrane γ secretase complex [4] [5]. NFTs are argyrophilic aggregates of hyperphosphorylated tau protein [6] [7]. Both protein aggregates display a β -pleated sheet conformation and are thought to interfere with cytoskeletal integrity, disrupt axonal transport, synapse and neuronal function. In a small portion of cases (<1%), the disease has an autosomal dominant pattern of inheritance termed familial AD (FAD), caused by mutations in one of three identified genes: APP, presenilin 1 (PS1), or presenilin 2 (PS2) [8]. Genotyping of tissue from the original histological slides from Deter's brain autopsy revealed she likely had PS1-linked FAD [9] [10]. In contrast to the original “amyloid cascade” hypothesis that $A\beta$ plaques drive the neuropathological cascade leading to dementia [11], Dr. Alzheimer wrote “...the plaques are not the cause of senile dementia, but only an accompanying feature of senile involution of the central nervous system” [12] [13]. In 1999, Mesulam [14] wrote, “It seems as if the $A\beta$ plaques appear at the wrong time and in the wrong places with respect to the clinical dementia and there is little evidence that they cause the NFT”. The revised amyloid cascade hypothesis posits that soluble $A\beta$ oligomers initiate the pathological cascade of AD leading to synaptic dysfunction, neuronal cell death, and dementia [15]. These findings support the contention that amyloid deposition is not the singular pathogenetic cause of sporadic AD.

AD is now thought to have an extensive preclinical stage, which is initiated 15-20 years prior to emergence of clinical signs (Fig. 2) [16, 17]. The clinical concept of mild cognitive impairment (MCI) arose from memory clinics. In the 1990's, some cases were characterized by an amnesic disorder and classified as amnesic MCI (aMCI) [18]. Although memory disorder clinics reported that aMCI was a more common form, MCI comprises a heterogeneous population: those with memory deficits only are referred to as single domain

aMCI, while those with a deficit in memory and another cognitive domain are termed multi-domain MCI (mdMCI) [19]. Individuals with aMCI are at a higher risk of developing AD [19]. MCI was initially characterized as an intermediate phase when NFT and A β lesions are increased relative to people with no cognitive impairment (NCI) [20] [21]. However, Accumulating evidence revealed that many clinically diagnosed NCI or MCI cases exhibit significant amyloid plaque (Fig. 3) as well as NFT pathology equal to or greater than what is often seen neuropathologically in mild to moderate AD, challenging the singular notion that these lesions alone drive dementia onset [21] [22] [23]. The demonstration that a cohort of NCI subjects who came to autopsy with high amyloid and NFT pathology support the developing concept of pathological aging or preclinical AD phase [24] [25].

Despite the extensive emphasis on amyloid and tau as the major factors leading to dementia onset and serving as the predominant focus of drug discovery and clinical trials, it is becoming evident that these lesions are not acting in isolation during the preclinical phases of AD [22] [26]. The fact that virtually every anti-amyloid clinical trial to date has not met its primary end-points of improving cognition [27] indicates that a monotherapy strategy may not be sufficient to treat this pervasive progressive multisystem neurodegenerative disorder [28]. On the other hand, it has been argued that AD is a disease of long projections neurons innervating the hippocampus and neocortex [26] [29], that degenerate early in the disease progression) [26] [29], which may disrupt brain connectivity and neuroplasticity. Although not receiving as much attention as amyloid and tau pathobiology, herein we review the molecular and cellular neuropathobiology of preclinical and prodromal AD. This information is essential for the development of appropriate therapeutic targets aimed at preventing or slowing cognitive decline prior to frank AD.

Amyloid and NFT pathology

A β peptides, which forms the core of parenchymal A β plaques are generated from APP, a holoprotein that is cleaved by successive β - and γ -secretases to form toxic A β [8]. The distribution of A β plaques changes with time and reflects the spread of A β deposition in the diseased brain [30]. Diffuse and “fleecy” plaques appear first throughout the neocortex and extend hierarchically into other brain regions [31]. In the next stages, A β plaques occur in allocortical areas, the basal ganglia, thalamus, hypothalamus, midbrain, medulla, pons and cerebellum. Later stages feature neuritic and dense cored A β plaques [32]. The other major AD pathological features are NFTs composed of aggregates of hyperphosphorylated forms of the tau protein (Fig. 1D, E) [33, 34]. NFT formation and maturation progresses according to a linear sequence of molecular and conformational alterations of the tau molecule [35]. Braak and Braak [32] described the topographic spread of NFTs from the mesial temporal lobe (MTL) to the neocortex, according to six stages depending upon location of the NFT-bearing neurons and the severity of lesions (transentorhinal stages I-II: clinically silent cases; limbic stages III-IV: incipient AD; neocortical stages V-VI: fully developed AD). Clinical subtypes of MCI as well as NCI cases display Braak staging scores ranging from stages 0-V [36]. These observations indicate that there may be no clear demarcation of neuropathology between some NCI, MCI and AD brains using the Thal staging criteria for amyloid [37] or the Braak staging criteria for NFTs [38].

NFT and cognition in preclinical AD

Although tau and A β pathology increase with age [39], their association with antimortem cognitive function remains relatively unexplored. NCI cases displaying AD neuropathology at autopsy despite a lack of clinically significant antemortem cognitive impairment may represent a pre-clinical disease stage [40]. Studies suggest a negative association between the presence of both A β plaques and NFTs with ante-mortem cognitive performance [30] suggesting that older individuals in the pre-clinical stages of AD according to their brain pathology at time of death continue to perform within normal limits on cognitive tests. Whether these individuals represent preclinical MCI remains an intriguing area of research.

While several groups have investigated the neuropathology of MCI and possible/probable AD, very few have concentrated on the relationship of Braak staging to clinical dysfunction in people with NCI [41]. An investigation of a small number of NCI subjects from the Rush Religious Orders Study (RROS) cohort revealed relatively similar percentages of low (40%) and high (60%) Braak scores compared to NCI, MCI and AD subjects examined [42] demonstrated a minor association between episodic memory and neuropathology using NIA-Reagan Institute neuropathological criteria for AD, which combines the assessment of neuritic plaques using CERAD scores and NFT pathology using Braak scores [43]. Individuals with low and high Braak scores and moderate to frequent CERAD scores displayed greater hippocampal and total brain volume loss suggesting brain volume as a variable that effects cognitive decline in the face of AD neuropathology [44]. Recently, we evaluated the association of low and high Braak NFT scores upon several cognitive domains {episodic, executive, semantic and a global cognitive score (GCS)} and plaque pathology in a cohort of elderly persons who were diagnosed as NCI at last clinical evaluation before death [22]. In this cross sectional investigation, the majority of the NCI subjects were Braak stage III (intermediate) and none were Braak stage VI (severe) (Fig. 4). Interestingly, females were significantly more likely to have higher Braak scores (III-V) despite being NCI, which is highly relevant due to the known gender differences associated with AD [45] [46]

Although the low Braak group of NCI performed better on most cognitive domains, only performance on semantic memory was significantly higher but this difference was not maintained when the data was adjusted for age, gender and years of education suggesting that this relationship was not directly related to Braak stage [36]. Hence, NFTs within the entorhinalperirhinal cortex (stages I–II), hippocampus (stages III–IV) and neocortex (stage V) may not be a necessary precondition for cognitive impairment in the elderly. We also evaluated whether Braak stage was associated with longitudinal change in the same cognitive domains [36]. Surprisingly, age but not Braak stage played a more prominent role in cognitive decline in NCI over time suggesting that the extent of NFT pathology does not directly influence cognitive decline in the elderly. Interestingly, NCI females, which displayed significantly greater NFT pathology (discussed above), performed worse than males on semantic memory and visuospatial domain tests, suggesting that NFT pathology influences cognitive decline in females to a greater degree than males. In addition, we found significant correlations between hippocampal CA1 neuritic plaques (NPs) and episodic memory or GCS [47] suggesting that this type of a lesion combining A β (plaque) and tau

(dystrophic neurites) pathologies plays a more critical role in cognitive decline during this preclinical stage of dementia.

Amyloid deposition and cognitive decline

A review of the literature suggests that cognition is affected by NPs and not diffuse plaques (DPs) [48] [49]. Greater hippocampal burden of NPs correlated with worse episodic memory and global cognitive performance and greater hippocampal/entorhinal cortex A β load was associated with greater NFT pathology in cases with an antemortem clinical diagnosis of NCI and a continuum of Braak stages [22]. In this same cohort, we found that NPs but not DPs were associated with significantly lower cognitive test performance across several cognitive domains [50], supporting previous reports [30, 51]. We also observed that NPs correlated with Braak stage stronger than DPs [30] [48]. Although we found that DPs were more prevalent than NPs in early Braak stages, NPs tended to increase and were nearly equal to DPs in higher Braak stages in NCI. Moreover, we found that in pathology burdened NCI, higher Braak stages were associated with greater amyloid plaque load in the entorhinal cortex but not in the hippocampus [47]. Group analysis found no significant effects of either hippocampal or entorhinal cortex amyloid load or APOE ϵ 4 status on Braak score. These findings demonstrate that people with NCI exhibit a wide range of Braak scores and plaque pathology similar to prodromal and frank AD.

Oxidative stress and synaptic homeostasis in the early progression of AD

Recently, we evaluated the relationship between oxidative stress and synaptic homeostasis in the early progression of AD [52] using NCI cases that may represent preclinical aMCI or preclinical AD (PCAD) from the University of Kentucky Alzheimer's Disease Brain Bank. Here, we compared age- and postmortem interval-matched hippocampus from NCI individuals who came to autopsy with high levels of AD pathology (HPNCI), low or no AD pathology (LPNCI) and an aMCI group for changes in both presynaptic (e.g., Synapsin-1, Synaptophysin) and postsynaptic (e.g., Drebrin, PSD-95 and SAP-97) proteins and markers of oxidative stress (e.g., protein carbonyls, 4-hydroxynonenal and 3-nitrotyrosine) (Fig. 5). Surprisingly, both the HPNCI and aMCI cohorts showed a significant decline in levels of the different synaptic proteins compared to LPNCI but aMCI and HPNCI groups did not differ significantly from each other (Fig. 5A, B). Measurements of oxidative stress also showed significant group differences with the greatest elevations observed in aMCI and HPNCI (Fig. 5C) and a significant association between changes in oxidative stress and synaptic proteins. The decline in both presynaptic- and postsynaptic proteins indicates a loss of synaptic homeostasis in the hippocampus early in the AD process. The increase in oxidative stress in aMCI was expected since numerous previous studies have emphasized such a scenario in the progression of the disease [53-56] Unexpected was that oxidative stress increased in the HPNCI cohort, further supporting the idea that this group may represent PCAD.

The factors underlying the decline in synaptic proteins in HPNCI cases are still to be determined. It may be related to changes in oxidative stress, since we found that levels of both pre- and postsynaptic proteins decreased as oxidative stress increased. Another possibility is that synaptic protein loss is associated with an increase in soluble A β ₁₋₄₂ (sA β ₁₋₄₂), which has been shown to increase during the progression of AD and is closely

associated with changes in cognition [57]. Examination of the role of sA β ₁₋₄₂ in the synaptic decline of the HPNCI group synaptic decline revealed that while the levels in aMCI were significantly higher than both NCI groups, there was no difference between the LPNCI and HPNCI, suggesting that the decline in synaptic proteins was unrelated to sA β ₁₋₄₂ levels (Fig. 5D). In addition, we found a significant association between CERAD scores and the levels of sA β ₁₋₄₂ in aMCI suggesting that while changes in sA β ₁₋₄₂ are probably downstream of synaptic changes, there are additional factors responsible for the transition from PCAD to aMCI.

During the last few years, oligomeric A β species have been suggested to be one of the triggering mechanism for the synaptic impairment and tau phosphorylation in AD [8, 58] [59]. Cortical levels of A β oligomers accumulate in MCI and mild to moderate AD compared to age matched non-demented controls [60], correlate with severity of cognitive impairment, Braak staging, and lower levels of presynaptic and postsynaptic proteins [61]. Oligomeric A β within synaptic terminals is associated with the accumulation of hyperphosphorylated tau in AD [59]. Interestingly, dimeric A β induces tau phosphorylation and dystrophic axonal profiles [62], suggesting a prion-like seeding or propagation mechanism underlying AD pathogenesis [63] or that selectively vulnerable subcortical long projection neurons may be sensitive to retrograde toxicity of A β oligomers. Therefore, therapeutic agents targeting synaptic A β oligomers early in the disease process may slow disease onset and trajectory.

Mechanisms delaying cognitive decline in non-cognitively impaired elderly

It is still unclear why some individuals with high AD pathology continue to perform well on cognitive tests. It is possible that NFTs and plaques are simply pathological bystanders and do not necessarily contribute to the initiation of cognitive dysfunction. Had these individuals lived longer they may transition to aMCI. Alternatively, one might consider the concepts of brain resilience and/or cognitive reserve as an explanation for this paradox [64] [65] [66] [67]. Brain reserve postulates a greater number of either neurons or synapses at the initiation of disease allowing the continued performance of cognitive tasks [65, 66]. On the other hand, cognitive reserve may involve the recruitment of brain regions either not or less severely affected by the disease process, which aid in task performance and even development of new brain connections [66]. Although the mechanism(s) initiating these constructs remain unknown, one mechanism may be related to brain neuroplasticity.

Brain Plasticity in MCI

The MTL memory circuit is highly neuroplastic [68] [69] and behaviorally resistant [22] to AD pathology. For example, similar entorhinal cortex amyloid loads were found between NCI, MCI and AD cases [47]. Studies using hippocampal tissue revealed a preservation of synapse number [70], synaptic protein [71], and neurotrophic factor receptors [72] in non-demented aged subjects with a wide range of Braak stages as well as an increase in dendritic spine size in prodromal AD [73]. Clinical pathological investigations also indicate preserved number and larger neuronal size in the hippocampus in “asymptomatic AD” compared with normal, MCI and clinical AD [74] indicating early reorganizational responses. A report revealed a significant increase in dendritic length (18%) and complexity (23%) in

hippocampal CA1 pyramidal in MCI compared to NCI individuals (Fig. 6A-C, F) and amyloid plaques distorted the dendritic architecture of CA1 neurons (Fig. 6E) [73]. Conversely, there was a significant reduction in branch length (–39%) and arbor complexity (–25%) during progression from MCI to AD (Fig. 5). Interestingly, increased synaptic contact size has been observed early in AD [75]. These types of early structural reorganization may represent a viable window for potential therapeutic strategies aimed at restoring or maintaining hippocampal function during the transition from NCI to MCI to AD.

In addition to structural alterations, there are biochemical neuroplastic responses, as well. For example, activity levels of choline acetyltransferase (ChAT), the rate-limiting synthetic enzyme for acetylcholine, are increased significantly in the hippocampus and superior frontal cortex in MCI compared to NCI or mild-moderate AD (Fig. 7A) [68] [69]. Across the three clinical diagnostic groups examined, increased hippocampal ChAT activity correlated with progression of hippocampal and entorhinal cortex NP pathology and limbic Braak stages [69], suggesting that this elevation is indeed a compensatory response to entorhinal-hippocampal disconnection (Fig. 7B, C). Further examination of the up-regulation of frontal cortical ChAT activity in MCI revealed that it was not paralleled by an increase in cholinergic fiber density in the same cortical region [76] suggesting that structural reorganization was not the mechanism activating this cholinergic plasticity response. It is more likely the result of a biochemical process that increases ChAT production within the cholinergic cortical projection neurons located within the nucleus basalis of Meynert [77], which is anterogradely transported to its cortical projection sites. Both the hippocampal and frontal cortex ChAT responses are transient [68], suggesting that this neuroplasticity is lost during the continued onslaught of the disease.

Another example of early neural reorganization is associated with entorhinal layer II glutamatergic neurons that innervate the hippocampus and degenerate early in the disease process [78]. It has been shown that alterations in glutamate receptor subunits precedes NFT formation in these two regions [79]. An investigation using specific immunological markers of glutamatergic neurons found a pathology-dependent pattern of glutamatergic synaptic remodeling within frontal cortex across the progressive phases of AD with MCI displaying an elevation in glutamatergic presynaptic bouton density, which depletes and is lost with disease progression [29]. Increased glutamatergic presynaptic bouton density correlated with improved cognition in AD, but not MCI, in which the increase in glutamatergic presynaptic bouton density was paradoxically related with decreased cognitive ability [29]. Perhaps this late chemical upregulation indicates a type of compensatory response intended to offset the effects of preexisting synaptotoxicity, or is an uncoordinated aberrant synaptic plasticity lacking functionality late in the disease. While neither interpretation has been confirmed experimentally, in light of the cholinergic hippocampal and frontal cortex reorganization findings and their association with better cognitive performance, the former seems more likely. Memantine, an FDA-approved partial antagonist of N-methyl-D-aspartate (NMDA) glutamate receptors, which acts to normalize glutamatergic neurotransmission has only modest symptomatic efficacy [80] [81]. New and more potent memantine-based drugs are needed to hopefully provide greater efficacy either alone or in combination with other compounds. In this regard, Namzaric, combines memantine hydrochloride extended-release

(Namenda) and donepezil hydrochloride (Aricept) an anticholinesterase which inhibits degradation of acetylcholine at the synapse. The extent to which other ascending neurotransmitter systems are capable of neuroplasticity during the progression of dementia remains an active area of investigation and a potential new set of drug targets.

Apolipoprotein E (ApoE) allele status, cognition and pathology

The genetic risk factor for AD, ApoE $\epsilon 4$, may also play a prominent role in the development of cognitive decline and pathology in AD [82]. Cognitively normal $\epsilon 4$ carriers are more likely to have significant AD-related pathology relative to non-carriers, but rates of cognitive decline are not significantly different between genotypes [83]. ApoE gene status, which mediates AD-related neuropathology, affects the onset and trajectory of cognitive decline [84] [85]. ApoE $\epsilon 4$ carriers display significantly greater amyloid load determined by Pittsburgh compound-B (PiB) imaging and show significantly lower cognitive test scores compared to non-carriers in non-demented elderly subjects [85] suggesting that APOE $\epsilon 4$ modifies the harmful effect of A β on cognition [86]. We found that soluble A β_{1-42} and fibrillary β -amyloid protein levels within the precuneus measured by PiB binding were significantly higher in AD than MCI and NCI, but were not associated with APOE $\epsilon 4$ status [87] [88]. Neuropathological reports show that AD pathology is limited within the precuneus suggesting a disconnection between amyloid toxicity and histopathology in this brain region [89] [88]. Perhaps this component of the default memory network is resilient to amyloid pathology [88].

Molecular signature of prodromal AD

A major area of interest in AD, is defining the molecular and cellular substrates of dementia onset. To accomplish this it is imperative to sample gene and noncoding RNA (ncRNA) expression from homogeneous brain cell types and correlate these changes with antemortem cognitive measures. Our group developed a strategy for the evaluation of individual genes that are differentially regulated as well as characterizing relevant classes of transcripts by gene ontology (GO) grouping during dementia progression using custom-designed microarray analysis following laser capture microdissection of immunohistochemically labelled neurons [90] [91]. For example, we observe a downregulation of transcripts during the progression of dementia in GO categories including glutamatergic neurotransmission, GABAergic neurotransmission, and synaptic-related markers [73, 90] [92] consistent with current hypotheses that synaptic degeneration, as well as deficits in excitatory and inhibitory neurotransmission underlie AD pathogenesis, which acting independent, or synergistically with amyloid deposits and NFTs [73, 92].

In contrast to observations of a gene downregulation in the majority of GO categories analyzed to date, significant upregulation of select endosomal-lysosomal pathway genes were found in CA1 pyramidal and cholinergic basal forebrain (CBF) neurons (Fig. 8). Quantitative analysis in CA1 pyramidal neurons indicated upregulation of early endosome effectors *rab4* (AD>MCI & NCI) and *rab5* (AD>MCI>NCI), late endosome constituent *rab7* (AD & MCI>NCI), and the trafficking molecule *rab24* (AD>MCI & NCI) [93]. Based on the comparison of expression level differences between MCI and AD, alterations in *rab5* and *rab7* were considered early, whereas *rab4* and *rab24* upregulation was considered a later

alteration [93]. A strong negative correlation was found between cognitive scores and *rab4*, *rab5*, *rab7*, and *rab24* CA1 neuron expression levels [93]. In addition, there was an upregulation of the acid hydrolase cathepsin D (*Ctsd*; AD & MCI>NCI), the early endosome antigen 1 (*Eeal*; AD & MCI>NCI), and lysosomal-associated membrane protein 1 (*Lamp1*; AD>MCI & NCI) [93]. In contrast, no differential regulation of rab GTPases that are not regulators of endosomes was observed across clinical groups [93]. Upregulated expression of *rab4*, *rab5*, *rab7*, and *rab27* in CBF neurons correlated with antemortem measures of cognitive decline in MCI and AD [94]. Results were validated by sub-regional qPCR and immunoblot analysis [93] [94].

Recently, we found upregulation of select endosomal-lysosomal genes, consistent with our previous single label analyses of vulnerable CBF neurons [94] at different stages of NFT evolution [23]. Pretangle-bearing CBF neurons (marked with the tau antibody pS422) [95] displayed a significant upregulation *rab5*, *rab7*, *Ctsd*, and *Lamp1* expression. Later stage NFT, visualized by dual-labeling immunohistochemistry using the pretangle, pS422 marker, and TauC3, a later stage tau neopeptide [96], was associated with *rab4* upregulation [23]. No expression level changes were found for amyloid-beta precursor protein (*APP*), amyloid-beta precursor-like protein 1 (*Aplp1*), amyloid-beta precursor-like protein 2 (*Aplp2*), *Bace1*, presenilin 1 (*Psen1*), presenilin 2 (*Psen2*), low-density lipoprotein-related protein 1 (*Lrp1*), high-density lipoprotein binding protein (*Hdlbp*), alpha-2-macroglobulin (*A2m*), and beta-2-microglobulin (*B2m*), among other AD-related genes [23] [92] [94]. The lack of expression changes related to amyloid and presenilin genes in these highly vulnerable neurons suggests that these mRNAs are not playing a major role in cellular deregulation early in disease process. Since deficits in the endocytic system is one of the earliest disturbances in AD and precede amyloid and NFT deposition as well as clinical symptoms of AD [97], dysfunction in this gene family may underlie, in part, the early selective vulnerability of hippocampal CA1 pyramidal and CBF neurons. Future studies using RNA sequencing (RNA-seq) at the single population level during the progression of dementia will likely enable the assessment of endosomal-lysosomal and other pathways for coordinate analyses through GO queries, as well as the ncRNAs that potentially regulate their expression.

The presence of small non-coding microRNAs (miRNAs) that negatively regulate mRNA stability [98] is an under researched area in AD [99, 100]. Various miRNAs regulate a multitude of brain functions (e.g., synaptic plasticity and energy metabolism) [101-104] suggesting that perturbations in miRNA function is involved in the pathogenesis of complex neurodegenerative disorders including AD [99, 100]. AD brains display altered expression of miRNAs that regulate BACE1 enzyme [105, 106] and are linked to tau phosphorylation [107-109] and pro-inflammatory actions [107, 110-112]. The role that the dysregulation of brain miRNA networks play early in AD are unclear. Our group using microarray and quantitative PCR analyses compared levels of miRNAs isolated from frontal cortex (FC) and inferior temporal cortex (ITC) tissue obtained from cases who died with a clinical diagnosis of NCI, aMCI, or mild AD [113]. Two families of miRNAs, miR-212/132 and miR-23a/b were down regulated in the FC in aMCI and AD compared to NCI, but were preserved in the ITC. Down-regulation of either group of miRNAs was predicted to increase the deacetylase Sirtuin 1 (*Sirt1*), which is involved in the mediation of protective neuronal cell stress responses [114]. *Sirt1* mRNA levels were greater in the aMCI FC but stable in ITC,

suggesting a connection between miR-212/132 and miR-23a/b down-regulation and decreased transcriptional repression of *Sirt1* expression. Experimental down-regulation of miR-212 and miR-23a *in vitro* up-regulated *Sirt1* resulting in neuroprotection against A β toxicity. These data suggest that up-regulation of *Sirt1* is a novel neuroprotective pathway activated early in the onset of AD.

Biomarkers during AD progression

Neuropathological examinations of older people who died with a clinical diagnosis of NCI or MCI often reveal similar pathological signatures to those with frank AD [40, 89, 115-118]. Hence, identification of individuals in the preclinical and prodromal stages of AD is essential for the timely administration of disease modifying therapies. Several initiatives have defined a panel of diagnostic and prognostic imaging as well as cerebrospinal fluid (CSF) and blood biomarkers that identify people at the earliest stages of the disease, with most fluid biomarker studies focusing detecting changes in CSF A β peptide and tau protein levels at varying AD stages [119]. The National Institute on Aging-Alzheimer's Association (NIA-AA) developed criteria for using biomarkers to determine the likelihood of AD pathology and for classifying patients accordingly [119, 120] which included CSF A β_{42} , positron emission tomography (PET) amyloid, CSF total tau, pT181 phospho-tau, MTL atrophy on Magnetic Resonance Imaging (MRI), tempoparietal/precuneus hypometabolism or hypoperfusion on PET or single-photon emission computed tomography (SPECT) [119]. Findings indicate that loss of hippocampal volume and the ratio of CSF A β_{42} to total tau or phospho-tau are predictive of longitudinal changes in cognitive measures [121-124]. Arterial spin labeling MRI is used to examine the influence of changes in resting cerebral blood flow as well as blood oxygenation level dependent signal response in relation to PET-derived regional amyloid load [125, 126] or to memory encoding in the MTL [127]. It has become increasingly clear that imaging radioligands, alone or in combination with other AD biomarkers will be critical for the earliest detection of AD pathology and timely initiation of therapy.

(³H)PiB PET imaging in NCI, MCI and AD

PiB 6-OH-BTA-1; [128]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole]] binds with a high affinity to β -sheet structured aggregates of fibrillar A β [129] and was the first PET imaging agent to differentiate AD from NCI (Fig. 9) [130]. It binds most prominently in NPs and vascular A β associated with cerebral amyloid angiopathy (CAA) [131], but does not bind to classic intracellular NFTs or other neuropathological elements [132]. *In vitro* [C-11]PiB binding across multiple brain regions distinguishes between clinical categories and correlates with fibrillar A β by enzyme-linked immunosorbent assay (ELISA) [133, 134], and correlates with antemortem [C-11]PiB PET retention [135]. Interestingly, 10%-20% of AD patients are PiB(-), in agreement with autopsy reports [136] [40], and 20-30% of NCI are also PiB(+)[137], which increases up to 65% in those >80 years [40, 138], suggesting a clinical misdiagnosis and/or a distinct dementia syndrome. Interestingly, APOE ϵ 4 is associated with higher PiB PET retention in elderly NCI [139], while in MCI it confers an increased likelihood of converting to AD [140]. The presence of A β deposits in PiB negative (-) subjects questions the sensitivity of this tracer [135].

The relatively short radioactive half-life of carbon-11 (~20 minutes), limits the distribution of PiB to PET imaging centers and supports the requirement of longer lived radioligands such as F-18 flutemetamol a 3'-fluoro analog of PiB (3'-F-PiB) [141], F-18 florbetapir [142] and F-18 florbetaben [143]. PET ligands for amyloid detection will be useful tools for the differential clinical diagnosis in large populations of individuals with suspected cognitive decline. To better define clinical states the field also needs tools to image tau profiles.

Tau PET imaging

Although research efforts have been successful in developing PET tracers that specifically bind to fibrillar A β pathology, a positive amyloid PET scan alone may not be sufficient for a positive diagnosis for AD. Since tau pathology in form of NFT burden is a better correlate of AD dementia, a PET tracer that selectively targets tau aggregates maybe a more relevant biomarker for AD conversion and for other tauopathies.[144] In normal brain there is an equal ratio of 3-repeat (3R) and 4-repeat (4R) tau isoforms, but in pathological states, tauopathies can express different isoform ratios with diverse morphologies, making it more difficult to develop a disease-specific tau PET tracer with similar affinity for every phenotype.[144, 145] In addition, tau undergoes several posttranslational modifications, resulting in conformational changes and excessive phosphorylation in the aggregates, potentially resulting in different tau ligand binding affinities. Since tau aggregates co-occur with A β deposits in AD, tau ligands need to be highly selective. During the past several years, several candidate tau PET imaging agents have been developed and used to image tau in different clinical phenotypes of AD (Fig. 10). [144, 146] Several groups reported tau-selective PET radioligands, including [F-18] labeled THK compounds [147] [148] [149-151] [152], PBB compounds [153] and [F-18] labeled T807 and T808 compounds [154] [155]. The latter compounds are the most widely used PET tracers for tau; they discriminated AD from control and non-AD dementias and demonstrated topographical patterns similar to neuropathological stages, suggesting their utility for assessing tau-related neuronal injury *in vivo* [150, 156, 157]. For example, in a study using ¹⁸F AV-1451 [155], patterns of cortical binding visualized with surface-projected SUVR thresholds were shown to be anatomically consistent with the ordinal stages of Braak pathology (0, I/II, III/IV, and V/VI) [38]. Similar to Braak stages 0, I, or II, many control cases displayed low overall or only mesial temporal lobe binding (Fig 10A–C), whereas greater neocortical binding was always associated with higher levels of temporal lobe binding in impaired cases (Fig 10D–G). The highest neocortical binding levels, consistent with Braak stages V–VI, were related with selective sparing of primary cortices (Fig 10F–G). These anatomic findings were confirmed quantitatively by voxel-wise and region of interest measures. ¹⁸F AV-1451 PET was found to be abnormally high in cortical, entorhinal, and parahippocampal regions (but not in the hippocampus) in MCI and AD compared to NCI, and greater radioligand retention in the inferior temporal gyrus correlated with impaired cognition [155]. Postmortem studies indicate that AV-1451 binds better to NFTs than A β plaques, but also identified off-target binding in some areas [158]. Other, less selective ligands also demonstrated good utility when compared to imaging of brain atrophy and glucose metabolism. For example, (¹⁸F)FDDNP (2-(1-{6-[159]fluoroethyl} (methyl)amino]-2-naphthyl}ethylidene)malononitrile), a lipophilic tracer, which binds to both A β plaques and NFTs [160] revealed increases in regional brain uptake which correlated with greater brain

atrophy on MRI and reduced FDG-PET [159]. Although (^{18}F)FDDNP PET and (^3H)PiB PET have similar retention patterns in neocortical regions, (^{18}F)FDDNP retention was greater in the mesial temporal lobe [161]. Higher global cortical uptake of (^{18}F) FDDNP PET and (^3H)PiB PET was seen in MCI and AD compared to NCI, however, (^3H)PiB proved better in detecting differences among the clinical groups [162]. (^{18}F)AV-1451 also has a promising pattern of retention in relation to clinical severity of AD [163], with greater PET retention in MCI and AD patients compared to NCI [154].

Taken together amyloid and tau PET imaging represent major advances in AD field, however there are still a number of limitations and unresolved questions. Sensitivity of PET imaging with [^{11}C]PiB and related [^{18}F] amyloid ligands is not well characterized for relatively low but histologically detectable A β deposits [164]. Analyses of large numbers of PET positive and PET negative cases, with short imaging-to-autopsy interval, are required to resolve these issues and establish threshold levels of A β and tau pathologies necessary for in vivo PET detection. A major challenge for neuroimaging in AD is how to determine the onset of amyloid and tau accumulation in pathology burdened NCI and MCI, and its association with cognitive measures and other biomarkers. Accordingly, the focus of PET imaging studies is currently shifting from AD towards NCI with the earliest fibrillar A β and tau deposits, to help determine the clinical significance of pre-symptomatic pathology and identify subjects at risk for cognitive decline. More studies are needed to directly compare the relative merit of amyloid and tau PET, MRI, FDG, clinical measures and CSF biomarkers.

CSF Biomarkers

Imaging-based technologies are relatively expensive and not readily transferred to community-based clinical settings, so fluid biomarkers are still desirable should the level of sensitivity and specificity for predicting conversion to dementia remain high. Panels of plasma inflammatory marker and related proteins [165], either alone or in combination with CSF biomarkers, have been proposed. CSF concentrations of A β_{1-42} and tau are indirect measures of AD pathology in the brain, with relatively high accuracy for identifying incipient AD [122] and predicting the development and rate of cognitive decline [123] [166]. In AD, CSF A β_{1-42} is reduced and phosphorylated tau is increased, which correlates with post-mortem amyloid and NFT pathology [167]. A strong concordance between PiB PET and CSF A β_{1-42} exists in NCI [168], and mixed cohorts of NCI and AD [169] [170], with no correlation between PiB PET and CSF tau [171]. Longitudinal studies suggested that amyloid PET may be more sensitive than CSF A β_{1-42} in identifying MCI cases that will convert to AD [172] [173]. A plasma phospholipid panel was recently identified that predicted conversion to MCI or AD within a 2-3 year timeframe with over 90% accuracy [174]. However, the inherent variability of AD neuropathology in the aged NCI, MCI and AD brain is also reflected by variation in these markers, particularly CSF and blood amyloid and tau [175], resulting in equivocal specificity and sensitivity for identifying at-risk individuals. Figure 11 shows a summary of data related to CSF biomarker diagnostic performance across a large number of primary studies published after the introduction of criteria recommended by the NIA-Alzheimer's Association Workgroups [176] showing the sensitivity, specificity, and likelihood ratios of CSF core biomarkers [177], and head-to-head

CSF biomarker performance based on average AD to control ratios [178]. Together in combination with clinical methods, cognitive tests and imaging, CSF biomarkers can contribute to the assessment of dementia and whether AD pathology is present in cognitively normal individuals [179]. Currently, there is a need to augment current CSF biomarker panels with novel proteins reflecting AD molecular pathogenesis to improve diagnostic accuracy in longitudinal studies assuming that AD is a multifactorial disease [180].

We discovered that protein levels of the precursor of nerve growth factor (NGF), termed proNGF, were selectively increased in postmortem neocortex [181, 182] and hippocampus [72, 183] of subjects who died with a clinical diagnosis of MCI or mild AD compared to NCI, respectively, which correlated with poorer performance on antemortem cognitive tests [181, 183]. Therefore, we tested whether CSF proNGF levels are an early marker of cognitive impairment using ventricular CSF (vCSF) obtained postmortem from the RROS cohort who came to autopsy with a clinical diagnosis of NCI, aMCI, or mild/moderate AD, and lumbar CSF collected from Washington University Knight AD Research Center subjects clinically diagnosed CDR 0 (no dementia), CDR 0.5 (MCI or very mild AD), or CDR 1 (mild AD) [184]. Quantitative western blotting of vCSF revealed a significant 55% increase in CSF proNGF levels obtained from aMCI compared to NCI and a 70% increase in AD compared to NCI, which showed a significant inverse association between increasing vCSF proNGF levels, cognitive deterioration and GCS [184]. Similarly, lumbar CSF samples revealed a significant 30% increase in proNGF levels in the CDR 0.5 and CDR 1 compared to CDR 0 cases. However, there were no differences in levels of $A\beta_{1-42}$, total tau, phospho-tau, or phospho-tau/ $A\beta_{1-42}$ among the groups, whereas the ratio of total tau/ $A\beta_{1-42}$ levels was 50% higher in CDR 1 subjects compared to CDR 0. To determine whether the inclusion of proNGF would improve the reliability of these biomarkers, we calculated proNGF/ $A\beta_{1-42}$, proNGF/total tau, and proNGF/phospho-tau ratios. Notably, proNGF/ $A\beta_{1-42}$ levels were 50% higher in CDR 0.5 and CDR 1 compared to CDR 0, whereas proNGF/total tau and proNGF/phospho-tau were not different between the groups. Our data suggest adding proNGF to a growing list of candidate biomarkers for AD. Increased CSF proNGF levels likely mirror the up regulation of this mainly proapoptotic protein seen in the neocortex and hippocampus in MCI and AD subjects, which we have suggested indicates a shift from cell survival to death [26]. Hence, we posit that increased CSF proNGF is an early pathobiological marker for disease onset. In sum, combining single or multiple tracer imaging using PET radioligands with CSF biomarkers will allow for earlier detection of AD, monitoring of pathology progression and effects of therapies, and selection of appropriate subjects for clinical trials of future AD therapies.

Concluding comments

Based on accumulating molecular, cellular, biochemical, neuroplasticity, neuroimaging, and clinical pathological findings, perhaps the field of AD research has reached a point where there is a need for a paradigm shift away from a strict interpretation of the amyloid hypothesis as it relates to the biological cascade of events that culminate in dementia. In his classic book “The Structure of Scientific Revolutions” the philosopher, Thomas Kuhn, proposed that intellectual progress is not gradual but is marked by sudden paradigm shifts [185]. The argument is made that there is a period when the field follows a paradigm that

has great promise such as the amyloid cascade hypothesis. However, during the last few years this hypothesis has been challenged by clinical pathological investigations demonstrating a large population of older individuals that display extensive pathological hallmarks of this disease, amyloid and tau lesions but do not develop dementia even in the eighth and ninth decades of life [22] suggesting that these pathologies alone may not be necessary or sufficient to initiate cognitive decline [186]. Whether these lesions are primary drivers of pathology, or pathological bystanders remains to be determined. The reviewed molecular, cellular, neuroimaging, and clinical pathological findings as well as the poor efficacy of amyloid vaccine clinical trials to date suggests that the disease process is more complicated than a singular hypothesis. We posit that it is time to move away from concentrating on a unitary bio-mechanistic trigger and subsequent mono-therapeutic approach to a more inclusive molecular mechanistic concept that involves not only amyloid and tau but the disconnection of vulnerable neuronal circuits related to attention, memory, and executive functions as well as the brain's neuroplasticity early in the disease process. This paradigm shift may lead to more diverse and multidrug treatment approaches for this most intransient neurological disease of aging.

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Highlights

1. Review of Pathophysiology of prodromal Alzheimer's Disease (AD)
2. Novel Biomarkers for AD: Imaging and CSF
3. Brain plasticity in prodromal AD
4. Review of literature of the neurobiology of Prodromal AD suggests a rethinking of amyloid hypothesis

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Figure. 1. Photomicrographs of (A) Oskar Fisher, (B) Alois Alzheimer, (C) Auguste Deter. (D) Neurofibrillary tangle visualized by the Bielschowsky silver method from the brain of Auguste Deter, (E) NFT containing the tau epitope Alz-50 and (F) Fluorescent image of a cored amyloid beta immunostained plaque (red) surrounded by a GFAP positive glia cell (green) in AD frontal cortex.

Figure. 2.
Diagram illustrating the extensive preclinical stage of Alzheimer's disease.

Figure. 3.

Photomicrographs showing the distribution of β -amyloid staining within the entorhinal cortex in MCI. A. Note that the crescent shaped band of β -amyloid staining extends to the border of the perirhinal cortex. B. Higher magnification of the region outlined in panel A by curved arrows. C. Higher magnification photomicrograph of densely stained β -amyloid plaques (black arrow in B). D. Cluster of neurofibrillary tangles within layer 2 of the EC (open arrow in B). Scale bar represents: A=800 μ m, B=200 μ m, C and D=40 μ m. (E) Histogram showing the adjusted β -amyloid loads for each NCI, MCI, and AD case examined. The means and standard errors are indicated above each diagnostic group. Adopted with permission from [47]

Graphs showing relationships between cognitive domains and Braak scores, APOE $\epsilon 4$ status and gender

Figure 4.

Linear graphs showing relationships between cognitive domains (A), Braak scores, (B) APOE $\epsilon 4$ status and (C) gender for the cohort of cases who died with clinical diagnosis of no cognitive impairment but post mortem neuropathological evaluation revealed amyloid and plaque pathology similar to those with AD dementia. Note the significant differences between male and female for semantic memory ($p=0.006$) and visuospatial ($p=0.04$) scores. Circles and triangles represent mean z-scores, error bars represent standard deviation. Adapted from with permission from [187]

Figure 5.

Scatter plots showing changes in different synaptic proteins within the hippocampus for each subject from the different cohorts: Low pathology no cognitive impairment (LPNCI), high pathology no cognitive impairment (HPNCI), amnesic mild cognitive impairment (aMCI). (A) Antibodies directed against presynaptic proteins synapsin-1 and synaptophysin are shown. (B) Antibodies directed against postsynaptic proteins Drebrin, SAP-97, and PSD-95. Horizontal lines indicate group medians. *p<0.05, **p<0.01, ***p<0.005, #p<0.001 compared to LPNCI. (C) Representative slot-blot for protein carbonyl, 4-hydroxynonenal, and 3-nitrotyrosine fractions from individuals classified as no cognitive impairment and low AD-like pathology (LPNCI), no cognitive impairment and high AD-like pathology (HPNCI), and amnesic mild cognitive impairment (aMCI). (D) Scatterplots showing the levels of soluble Aβ₁₋₄₂. No significant difference were observed between the two groups with individuals labeled as NCI (p > 0.1). However, both the low pathology and high pathology NCI groups were significantly lower than the aMCI cohort. *p<0.05. Circles=LPNCI, Squares=HPNCI, Triangles=aMCI. Adapted from with permission from [188]

Figure. 6.

Golgi impregnated hippocampal CA1 pyramidal neurons analyzed for dendritic branching of the basilar tree from cases with a clinical diagnosis of (A) no cognitive impairment, (B) mild cognitive impairment and (C) Alzheimer's disease. (D) Note that amyloid deposit (*) distorts the dendritic architecture of a CA1 dendrite. (E) Scholl analysis of the amount, distribution and complexity of the arbor showed a significant increase in these parameters (length, + 18%; complexity, +23%) in MCI CA1 neurons compared to NCI. Conversely, there was a significant reduction in branch length (−39%) and arbor complexity (−25%) in the progression from MCI to AD. Adopted from with permission from [22]

Figure 7.

(A) Choline acetyltransferase (ChAT) activity increased in the hippocampus in MCI and returned to control levels in mild AD. (B and C) Schematic drawings of coronal section of the hippocampus illustrating the loss of innervation arising from the glutamatergic layer II entorhinal cortex neurons (red) triggering a cholinergic plasticity response (blue) in the MCI hippocampus. Abbreviations: AD: Alzheimer's disease; CA1, CA2, CA3, CA4- *Cornu Ammonis* hippocampal subfields; CS- collateral sulcus, DG-dentate gyrus, Ent-entorhinal cortex; f-fornix, gl-granular cell layer, ml- molecular layer; NCI- no cognitive impairment, MCI-mild cognitive impairment, pp-perforant pathway, TEC- transentorhinal cortex, Sub-subiculum.

Figure 8.
 (A) Heat map and histograms (B) showing changes in endosomal-lysosomal and nerve growth factor receptor gene levels during the progression of AD.

Figure 9. Representative images of $[C-11]PiB$ PET in subjects with NCI, MCI, or AD. Some NCI and MCI subjects have positive $[C-11]PiB$ PET scans (NCI+, MCI+) indicating an ongoing process of brain amyloidosis. Image courtesy of the University of Pittsburgh Amyloid Imaging Group.

Figure 10.

Cortical patterns of ^{18}F -T807 binding. Coronal ^{18}F T807 positron emission tomographic (PET) images (top row) and whole-brain surface renderings of standardized uptake value ratio (SUVR; cerebellar reference; second row) from 3 clinically normal (CN) and 4 impaired (2 mild cognitive impairment [MCI] and 2 mild Alzheimer dementia [43] dementia) participants. Top: (A) A 71-year-old CN subject with low amyloid β ($\text{A}\beta$) by Pittsburgh compound B (PiB) PET (mean cortical distribution volume ratio [DVR]=1.0) had low, nonspecific ^{18}F T807 binding in cortex, consistent with a Braak stage less than III/IV. (B) A 74-year-old CN subject with high $\text{A}\beta$ (DVR=1.2) with ^{18}F T807 binding in inferior temporal cortex, left>right, consistent with Braak stage III/IV. (C) A 79-year-old CN subject with high $\text{A}\beta$ (DVR=1.8) had binding in inferior temporal neocortex, consistent with Braak stage of III/IV. B and C show focally intense subcortical uptake that is likely due to off-target binding. (D–G) Cognitively impaired participants all with high $\text{A}\beta$ and with successively greater levels of cortical ^{18}F T807 binding successively involving temporal, parietal, frontal, and occipital cortices. Bottom: ^{18}F T807 SUVR calculated at vertices indicating the extent of cortical binding, with left hemisphere views (lateral, inferior, superior, medial) at left. The 52-year-old AD dementia patient (G) showed confluent ^{18}F T807 binding that is nearly pancortical, sparing only portions of primary cortex and consistent with Braak stage V/VI. Dx=classification; MMSE=Mini-Mental State Examination; PET Braak=estimate of Braak stages based on the anatomic pattern of T807 binding assessed visually and quantitatively in regions and full volume data. Legend and figure are reproduced with permission from *Annals of Neurology* Volume 79, Issue 1, pages 110-119, 15 DEC 2015 DOI: 10.1002/ana.24546 <http://onlinelibrary.wiley.com/doi/10.1002/ana.24546/full#ana24546-fig-0001>.

Figure 11.

Summary of CSF biomarker diagnostic performance. A) Sensitivity, specificity, and likelihood ratios of CSF core biomarkers based on primary studies published after the introduction of criteria recommended by the NIA-Alzheimer's Association Workgroups* [176]. B) Head-to-head CSF biomarker performance based on average AD to control ratios. Biomarkers are differentiated based on significant differences with good effect sizes (purple), significant differences with moderate effect sizes (purple), or non-significant or significant differences with minor effect sizes.** *Reprinted from *Frontiers in Aging Neuroscience*, Ferreira et al., Meta-review of CSF core biomarkers in Alzheimer's disease: the state-of-the-art after the new revised diagnostic criteria, pp. 1-24, 2014 (Open Access). [177]. *Reprinted from *The Lancet Neurology*, Olsson et al., CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis, doi:10.1016/S1474-4422(16)00070-3 2016, with permission from Elsevier. [189]