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(1) Clinical utility of the pathogenesis-related proteins in Alzheimer's disease

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Clinical utility of the pathogenesis-related proteins in Alzheimer's disease

Abstract

The Aβ cascade and alternations of biomarkers in neuro-inflammation, synaptic dysfunction and neuronal injury followed by Aβ have progressed. But the question is how to use the biomarkers. Here, we examine the evidence and pathogenic implications of protein interactions and the time order of alternation. After the deposition of Aβ, the change of tau, NfL and NG is the main alternation and connection to others. The neuro-inflammation, synaptic dysfunction and neuronal injury function is exhibited prior the structural and metabolic changes in the brain following Aβ deposition. The time order of such biomarkers compared to the tau protein is not clear. Despite the close relationship between biomarkers and plaque Aβ deposition, several factors favor one or the other. There is an interaction between the proteins that CSF SNAP-25, VILIP-1 and YKL-40 can predict the brain amyloid burden. The Aβ cascade hypothesis could be the pathway, but not all subjects are converted to AD, even with very high elevated Aβ. The interaction of biomarkers and the time order of change require further research to identify the right subjects and right molecular target for precision medicine therapies.

Keyword: Alzheimer's disease, biomarkers dynamics, interaction, time order

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Introduction

The amyloid hypothesis of Alzheimer's disease (AD) proposes that accumulation of amyloid-beta (Aβ) in the brain triggers pathogenesis of AD and the cascade of the spread of tau-related neurofibrillary tangles, neuro-inflammation, and neuronal degeneration. The failure of the anti-Aβ clinical trials indicated that the therapeutic potential of BACE-1 inhibition and anti-amyloid antibody is doubling [1-4]. The question on the amyloid hypothesis of Alzheimer's disease has been raised in recent years.

However, much evidence still confirms that the Aβ appears to be the initial disease mechanism in AD. Among initially amyloid-negative adults, Farrell found a regionally specific association between declining episodic memory and increased amyloid accumulation across multiple posterior cortical regions [5-6].

In the event of failure of clinical trials, Knopman[1] considered the selection of a population with positive Aβ to be too late for maximum treatment effect. Bischol[7] and Palmqvist[6] have proposed a potential solution for enrolling subjects with threshold amyloidosis. From experience, defining the threshold is very difficult. Higher SUVR of Aβ does not always indicate the late AD stage. At the same level of Aβ PET, higher FDG-PET reduces the risk of AD in MCI or Normal subjects. Even in the subjects with very high SUVR of Aβ, the modulation of higher FDG-PET continues to reduce the risk of conversion to AD (zhou, unpublished).

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Many studies have shown that in the downstream of Aβ, proteins have changed the spread of tau-related neurofibrillary tangles, synaptic dysfunction, neuro-inflammation, neuronal injury and neuronal degeneration all of which are associated with the pathogenesis of AD. However the interaction and the time order of changes of the proteins are not clear yet, which makes it difficult to clarify the molecular pathway. Palmqvist [6] proposed one order of the biomarkers based on the accumulation of Aβ PET in a cross-sectional data that could not reflect the time order. As noted by the author, it requires the follow-up of Aβ PET and the rates of change of Aβ.

In the hypothesis of AD pathogenesis proposed by Jack CR [8], the change of MRI and PDG-PET is accompanied by the accumulation of Aβ. Some studies showed that accumulation of Aβ resulted in hippocampal atrophy. However, the deposition of Aβ does not imply that the hippocampus is definitely atrophy. Aβ pathology and hippocampal atrophy are independently associated with memory function in cognitively healthy elderly people [9]. In the prediction analysis, MCI or normal cognition subjects with positive Aβ and hippocampal atrophy increased the risk of transition to AD. We also observed that not all subjects with elevated Aβ have hippocampal atrophy. The change in glucose metabolism measured by FDG-PET showed similar results. The involvement of structural MRI and glucose metabolism measured by FDG-PET with the pathogenesis of AD can be independent of Aβ. Successful treatment strategies can be devised by understanding the contribution of these markers to different aspects of disease pathogenesis.

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The framework of amyloid (A), tau (T), and neuro-degeneration (N) biomarkers is proposed to define the state of patients with regard to Alzheimer pathologic change. The group with abnormal levels of amyloid, tau, and neuro-degeneration (A+T+N+) showed consistently greater cognitive decline than the group with normal levels of all biomarkers (A-T-N-)[10]. The ATN system has different implications in patients with vs without dementia that it poses a challenge to discriminate between patient populations with specific features [11] and clinical trial design. Timing is always the challenge for subjects enrollment in AD clinical trials [1,12]. Before we figure out the pathway that includes pathological molecular changes and related association, we cannot make a clear definition of the timing. Based on the published results, efforts shall be made to clarify the interactions and time order of the proteins' change in order to set up the pathway of AD pathogenesis, as shown in Figure 1.

1. The change of biomarkers in Alzheimer's Disease

   1.1 Aβ and tau

   Researches indicated that CSF Aβ decreased while Brain PET Aβ increased in AD when compared with CN. CSF t-Tau and CSF p-tau increased at an early stage of the AD time pathogenesis compared to mild cognitive impairment (MCI) and normal cognition group [13-15]. Longitudinally, CSF Aβ decreased in all groups, however t-tau and p-tau levels increased significantly in the CN+ or CN group, while it decreased in the AD+ or AD group [13-14].

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1.2 Synaptic dysfunction

Synaptic dysfunction and degeneration are early fundamental pathophysiological characteristics of AD. Biomarkers that can track synaptic dysfunction in AD are eagerly awaited.

The major proteins of synaptic dysfunction involved in the pathogenesis of AD are neurogranin, SNAP-25, synaptotagmin, syntaxin and Ca2＋. complexin-1, complexin-2.

CSF Ng was increased in AD compared to CN or Parkinson's disease frontotemporal dementia and amyotrophic lateral sclerosis [15-17]. Baseline levels of Ng were significantly higher in the AD+ group than the CN and MCI− groups. The levels were also higher in the MCI+ group compared to the Aβ- (CN and MCI) groups. Longitudinally, CSF Ng or Brain Ng significantly decreased in the AD+ group or AD patients [13-14]. Ng/BACE1 levels were elevated in both subjective cognitive decline and mild cognitive impairment compared to healthy controls [18], and can distinguish between depression and AD among patients with similar cognitive deficits, along with the classic AD biomarkers [19].

SNAP25 increased in AD or MCI versus CN [12,15]. However Beeri [20] and Shimohama [21] indicated dementia to be associated with reductions in SNAP-25. Longitudinally, SNAP-25 levels declined significantly in the AD+ group, which indicates the varying level of SNAP25 at different stages of AD.

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In AD, the levels of synaptobrevin and synaptophysin decreased significantly from the levels of control. Other synaptic factors of synaptotagmin, and syntaxin 1/HPC-1, complexin-1, complexin-2 and septin-5 have also been reported to decrease [20-21].

1.3 Neuro-inflammation

The inflammation associated proteins include microglial function, TREM2, YKL-40, IP-10, complement C etc.

Chitinase-3-like protein 1 (CHI3L1), also known as YKL-40, is a secreted glycoprotein that is approximately 40kDa in size and is encoded by the CHI3L1 gene in humans. Elevated concentrations of YKL-40, VILIP-1 and sTREM2 in CSF were observed in MCI when compared with the control [22]. Baseline YKL-40 was significantly higher in the AD+ when compared with the MCI−. Longitudinally, YKL-40 in all Aβ+ and Aβ- groups of AD, MCI, and CN showed an increase in mean levels over time [13]

Using post-mortem human, Singh-Bains [23] demonstrated that there was 91% and 69% increase in the expression and load of IBA1(microglia) , respectively; the process length and branching of HLA-DR(microglia) positive cell was reduced by 33% and 49%, respectively; there was a 27% increase in the Astrocytes (GFAP) basement-membrane associated molecules Fibronectin expression in AD. Microglial and neurovascular dysfunction act as drivers of AD. In the case of TREM2, Plasma sTREM2

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does not differ between healthy controls, mild cognitive impairment (MCI) or AD. [24]

1.4 Neuronal injury

Cerebrospinal fluid (CSF) neuro-filament light (NFL) is a protein biomarker of axonal injury. Visinin-like protein 1 is a protein encoded by the VSNL1 gene in humans. This gene is a member of the visinin/recoverin subfamily of neuronal calcium sensor proteins. NF-L and VILIP-1 of neuronal injury have been reported to predict more FTD disease progression than AD.

Synaptic damage, axonal neuro-degeneration, and neuro-inflammation are common features in Alzheimer's disease (AD), frontotemporal dementia (FTD), and Creutzfeldt-Jakob disease (CJD). Although there was a stepwise increase in CSF NFL levels between control participants, participants with MCI, and Alzheimer disease; however the concentrations of NFL were highest in participants with amyotrophic lateral sclerosis and frontotemporal dementia (FTD)[25]. Researches indicated Neuronal injury related biomarkers of YKL-40 and NfL are valuable tools for staging and predicting patients within the ALS-FTD clinical spectrum [26], or as a disease progression biomarker in genetic frontotemporal dementia [27-28] or in distinguishing behavioral variant frontotemporal dementia (bvFTD) from primary psychiatric disorders (PSY) [29].

cNfL has the potential to assist in the differentiation of FTD from AD and

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PD from atypical parkinsonian syndromes [30]. NFL is not a specific biomarker for the diagnosis or progression of AD.

Mavroudis indicated that VILIP-1 levels are significantly higher in AD compared to normal controls, and these levels are significantly higher in patients with MCI progressed to AD, than in stable MCI patients in a meta-analysis [31]. As CSF VILIP-1 is an unspecific marker for neuronal injury and CSF myelin basic protein reflects neuroaxonal, the results of longer periods of stress associated with higher levels of CSF VILIP-1 suggested that long term stress may be associated with neurodegenerative processes in the brain [32].

The correlations between microglial activation with tau or amyloid deposition were stronger in Alzheimer's disease than in mild cognitive impairment, suggesting that these pathologies increase together as the disease progresses [33].

2. The interaction between the proteins and association with neuroimaging findings

2.1 Interaction between the proteins

Based on the Aβ cascade hypothesis, the deposition of Aβ resulted in the change of the biomarkers. Beyond the levels of individual proteins, protein-

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protein interactions that are critical to the process of vesicular neurotransmission also contribute [34]. Identifying protein interactions may make it easier to understand the pathogenesis and early prevention of AD.

55% of MCI and 83% of AD subjects had a high Aβ load [36] . NFL and YKL-40 were associated with Aβ+ in non-demented individuals only [37]. CSF YKL-40 was positively associated with both CSF NFL and T-Tau [38]. Jin M [39] reported that CSF NFL levels correlated with total tau, phosphorylated tau, and neurogranin but not with beta amyloid (Aβ).

Baseline CSF t-Tau levels were significantly elevated in the AD+ group when compared with Aβ+(MCI CN) and Aβ-(MCI and CN) groups. Longitudinally, t-Tau levels significantly increased in both the CN and MCI+ groups, but decreased in the AD+ group. VILIP-1, SNAP-25 increased significantly with Aβ in the Aβ+ of MCI and AD when compared with Aβ- groups [13].

For the association of inflammation with Aβ, microglial activation is positively associated with tau aggregation in MCI and AD, while negatively associated with amyloid deposition [33]. Levels of Aβ and inflammation (11C-(R)-PK11195) can be seen related in MCI. There was no detected association between Cortical tau tangles and inflammation in high amyloid-β cases [36].

There was difference in Ng and T-tau between Aβ+ and Aβ- individuals in each clinical group [37]. In brain tissue from patients with familial and sporadic

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Alzheimer's disease, Ng was significantly associated with the degree of amyloid and tau pathology [40]. Higher levels of SNAP-25-syntaxin interaction (controlled for the level of SNAP-25 as well as for pathology) were associated with lower likelihood of dementia. An association between SNARE complex formation and cognitive function that is in part regionally specific, and may be more prominent for specific cognitive domains [34].

Multiple variables were differentially associated with CSF NfL and T-tau levels, but not Ng. Most associations were attenuated after adjustment for age and sex. T-tau had the strongest association with cognition in the presence of amyloidosis; followed by Ng. Variables associations with NfL did not differ by amyloid status [38].

Although there is inconsistency in the results, majority of the results indicated that for biomarkers of t-Tau, P-tau, VILIP-1, SNAP-25, Ng, NFL, YKL-40 and inflammation (11C-(R)-PK11195), microglial activation began to change after the deposition of Aβ. NFL is also reported to be correlated with total tau independent of Aβ. The data of the association among other biomarker is still limited and it is difficult to identify interactions between biomarkers except for Aβ (Figure 2)

2.2 Association of the proteins with the neuroimaging findings and phenotypes

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We know that change in the brain structure can be measured by MRI Metabolism using FDG-PET after the deposition of Aβ in brain. However when and how the biomarkers are associated with the neuroimaging findings will be discussed in the next few paragraphs.

In amyloid-β-positive individuals, the atrophy across the entire brain is correlated with a summary measure of medial temporal lobe (MTL) 18F-AV-1451 uptake [41]. Aβ aggregation within the brain's default mode network leads to regional hypo-metabolism, and an interaction between this hypo-metabolism with overlapping Aβ aggregation is associated with subsequent cognitive decline [42].

For the synaptic biomarkers, in the temporal lobe, the trimeric SNARE protein interaction (SNAP-25, syntaxin, VAMP) was associated with the rate of cognitive decline and global cognitive function [34]. SNAP-25 genotypes also correlate with a significantly decreased brain activity in the cingulate cortex, and in the frontal and temporo-parietal area [43]. Elevated SNAP-25/ Aβ42 ratio or higher Ng/BACE1 ratio was associated with the rate of hippocampal atrophy in pMCI and the rate of change of cognitive impairment in CN over the follow-up period [44-46]. A research also showed an association between longitudinal decline in white matter microstructure and change of Aβ42, phosphorylated-tau, total-tau, NFL, and neurogranin [47].

The levels of individual CSF biomarkers of visinin-like protein 1, neurogranin,

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BACE1, Aβ1-40, Aβ1-38, and YKL-40 were all inversely correlated with the volume of gray matter of the precuneus in cognitively intact older subjects [48].

In inflammation, greater MCP-1, lower Aβ42, and greater P-Tau181 was associated with altered microstructure in bilateral frontal, right temporal lobe, and microstructure in precuneus, respectively [37]

Plasma and CSF NFL is cognitive in MCI [49-51] and positively with atrophy [50-51], hypometabolism, CSF biomarkers, and injury [50]. Serum NfL is not associated with amyloid-β deposition or glucose metabolism [51]. Elevated NFL predicted white matter damage in cognitively impaired older adults who are amyloid-negative and tau-positive [52]

3. The time order of the changes of the biomarkers

The time order of some biomarkers change is as follows CSF Aβ→CSF P-tau→CSF T-tau→PET Aβ-→PET tau. However we have no knowledge of when the changes occurred for other biomarkers and the time location with the MRI findings and glucose metabolism.

Only after Aβ PET became abnormal were the biomarkers of neuroinflammation, synaptic dysfunction, and neurodegeneration altered. Many findings provide *in vivo* support of the amyloid cascade hypotheses in humans [6]. Aβ42 caused the Cortex atrophy [13]. Brain amyloid deposition

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shortly caused early change of CSF tTau, pTau, SNAP-25, VILIP-1, and YKL-40. At the same time the change of CSF SNAP-25, VILIP-1, and YKL-40 also indicated performance on a cognitive composite, brain Aβ, 15-19 years before the estimated years from the onset of symptom [53]. CSF-amyloid is more sensitive early in the course of the disease.

In an animal model of AD, Ng levels increased in CSF when neuro-degeneration was induced, peaking after 2 weeks, while it decreased in a brain when CSF Ng is a biomarker of synaptic degeneration [17].

The change of NfL appeared before the change of structural MRI findings and glucose metabolism measured by FDG-PET. In Aβ+ participants, NfL associates with hypo-metabolism in AD-vulnerable regions [54]. Merluzzi suggest that NFL may be more sensitive to subclinical cognitive decline compared to other proposed biomarkers for neurodegeneration, whereas neurogranin and t-tau are not [55].The rate of change of serum NfL could distinguish mutation carriers from non-mutation carriers almost a decade earlier than cross-sectional absolute NfL levels (16.2 versus 6.8 years before the estimated onset of AD symptom) [51]. Across all mutation carriers, serum NfL correlated with EYO（estimate　year of onset）and multiple cognitive and imaging measures [56]

However biomarker change is not always associated with Aβ. Increased concentrations of baseline plasma t-tau predicted structural basal forebrain

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cholinergic system (BFCS) atrophy progression in older adults at risk of AD, independently of β-amyloid status and APOE genotype [57]. Elevated CSF NfL levels but not CSF T-tau, P-tau or Neurogranin are a risk factor for MCI in a community population, and they are independent of brain amyloid [58]. Rate of change of serum NfL was more associated with cortical thinning but less with amyloid-β deposition or glucose metabolism (assessed by positron emission tomography)[51]

Researches have shown that cerebrovascular processes are involved in the onset of the pathogenesis of AD, in which Cerebral amyloid angiopathy (CAA) is one of the factors. Accumulating evidence indicated that the intersections between CAA and AD has a crucial role to play in improving vascular function in the treatment of both diseases and indicated the next steps needed to identify therapies [59]. Cilostazol, a selective inhibitor of phosphodiesterase (PDE) III, promoted perivascular drainage of soluble fluorescent Aβ1-40 in Tg-SwDI mice [60]. However, the clinical trial of Cilostazol on the prevention of MCI is ongoing [61].

Conclusion

We observed that the deposition of Aβ resulted in the change of the biomarkers related to synaptic function, neuro-inflammation and neural injury, in which NfL and NG are majorly alternating. The relationship with tau protein is not consistent. Some research showed that alternation occurred before tau protein; but the relationship and time order of such biomarkers with tau protein

is not clear. The biomarkers experienced changes prior changes of the MRI findings and metabolism. The Aβ cascade could be the main hypothesis, however not all subjects converted to AD even with very high elevated Aβ. The interaction of biomarkers and the time order of the change require further research to identify the right subjects and right molecular target for precision medicine therapies.

Author Contributions

Conceptualization, Bin Zhou: Investigation, Bin Zhou; Original Draft Preparation, Bin Zhou; Writing – Review & Editing, Bin Zhou, Masanori Fukushima; Supervision, Masanori Fukushima;.

Conflicts of Interest

The authors have no conflicts of Interests to be disclosed

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Figure1 Hypothesis of AD pathogenesis

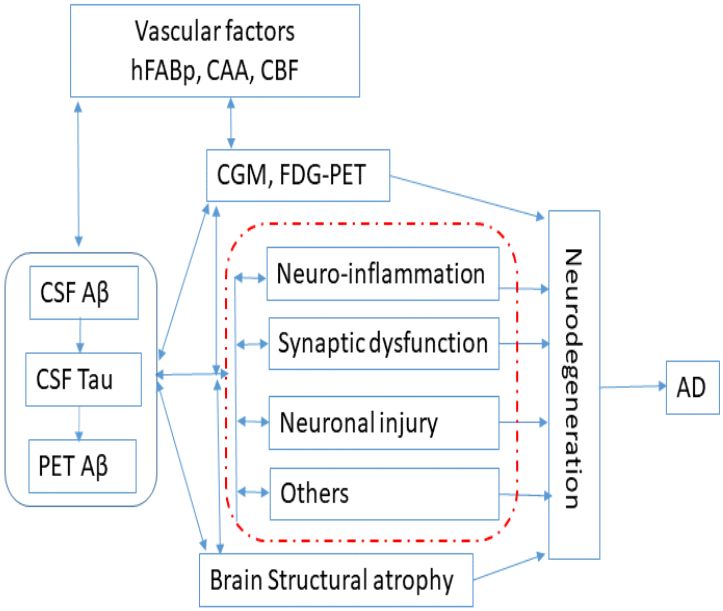

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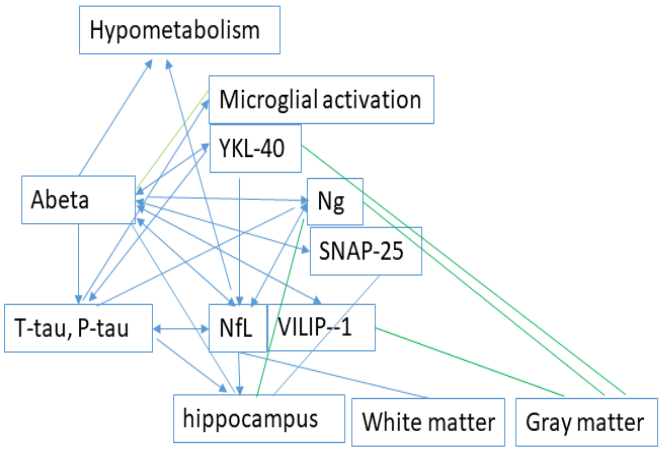
Figure2 Interaction among the proteins



Blue: association; Green: Negative association;