Monitoring synaptic pathology in Alzheimer's disease through fluid and PET imaging biomarkers: a comprehensive review and future perspectives

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ABSTRACT

Alzheimer's disease (AD) is currently constrained by limited clinical treatment options. The initial pathophysiological event, which can be traced back to decades before the clinical symptoms become apparent, involves the excessive accumulation of amyloid-beta (Aβ), a peptide comprised of 40−42 amino acids, in extraneuronal plaques within the brain. Biochemical and histological studies have shown that overaccumulation of Aβ instigates an aberrant escalation in the phosphorylation and secretion of tau, a microtubule-binding axonal protein. The accumulation of hyperphosphorylated tau into intraneuronal neurofibrillary tangles is in turn correlated with microglial dysfunction and reactive astrocytosis, culminating in synaptic dysfunction and neurodegeneration. As neurodegeneration progresses, it gives rise to mild clinical symptoms of AD, which may eventually evolve into overt dementia. ~~Preclinical studies have pinpointed synaptic loss as a major pathological factor associated with cognitive impairment in AD, potentially indicating early changes in disease pathophysiology, possibly transpiring even prior to alterations in tau function.~~ Synaptic loss in AD may develop even before tau alteration and in response to possible elevations in soluble oligomeric forms of Aβ associated with early AD. ~~However, t~~These findings largely rely on *post-mortem* autopsy examinations, which typically involve a limited number of patients. Over the past decade, a range of fluid biomarkers such as neurogranin, α-synuclein, visinin-like protein 1 (VILIP-1), neuronal pentraxin 2, and β-synuclein, along with positron emission tomography (PET) markers like synaptic vesicle glycoprotein 2A, have been developed. These advancements have facilitated the exploration of how synaptic markers in AD patients correlate with cognitive impairment. However, fluid biomarkers indicating synaptic loss have only been validated in cerebrospinal fluid (CSF), not in plasma, with the exception of VILIP-1. The most promising PET radiotracer, [^{11}C]UCB-J, currently faces significant challenges hindering its widespread clinical use, primarily due to the necessity of a cyclotron. As such, additional research geared towards the exploration of synaptic pathology biomarkers is crucial. This will not only enable their extensive clinical application, but also refine the optimization process of AD pharmacological trials.

LIST OF ABBREVIATIONS

[^{18}F]-FDG-PET, [^{18}F]-fluorodeoxyglucose-positron emission tomography; α-syn, alpha-synuclein; β-syn, beta-synuclein; Aβ, amyloid beta; ~~Aβ-, Aβ PET negative; Aβ+, Aβ PET positive;~~ $A\beta_{1\text{-}42}$, 42-amino acid-long Aβ peptide; $A\beta_{1\text{-}40}$, 40-amino acid-long Aβ peptide; AβOs, soluble Aβ oligomers; AD, Alzheimer's disease; ADAD, autosomal-dominant AD; ADNI, Alzheimer's Disease Neuroimaging Initiative; AI, artificial intelligence; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPARs, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; APP, amyloid precursor protein; CaM, calmodulin; CaMKII, calcium/calmodulin dependent protein kinase II; CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; DIAN, Dominantly Inherited Alzheimer Network; EEG, Electroencephalography; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration; GAP-43, growth-associated protein-43; HCs, healthy controls; LBD, Lewy bodies dementia; LOAD, late-onset AD; LTD, long-term depression; LTP, long-term potentiation; MCI, mild cognitive impairment; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MEG, magnetoencephalography; mGluR5, metabotropic glutamate receptor 5; ML, machine learning; MMSE, Mini-Mental State Examination scale; NfL, neurofilament light chain; NFTs, neurofibrillary tangles; Ng, neurogranin; NCS, neuronal calcium sensor; NMDA, N-methyl-D-aspartate; NMDARs, N-methyl-D-aspartate receptors; NPTX2, neuronal pentraxin 2; NPTXR, neuronal pentraxin receptor; NPTXs, neuronal pentraxins; NRG1, neuregulin 1; NRN1, neuritin; Nrxs, neurexins; NLs, neuroligins; p-tau, hyperphosphorylated tau protein; $p\text{-}tau_{181}$, tau hyperphosphorylated at threonine 181; PD, Parkinson's disease; PET, positron emission tomography; PH, prominent h-current; RBCs, red blood cells; SCD, subjective cognitive decline; SNAP-25, synaptosomal-associated protein, 25kDa; SNARE, Soluble N-ethylmaleimide-Sensitive Factor Attachment Proteins Receptors; SV2A, synaptic vesicle protein 2A; t-tau, total tau; TMS, transcranial magnetic stimulation; UCSD, University of California San Diego; VILIP-1, visinin-like protein 1; WHO, World Health Organization (WHO). ~~WT, wild-type.~~

INTRODUCTION

Alzheimer's disease (AD), a progressive neurodegenerative disease (ND), evolves through distinct stages – starting with an extended asymptomatic period of cognitive normalcy (preclinical AD), transitioning into a prodromal symptomatic phase of mild cognitive impairment (MCI), and eventually culminating in clinically overt dementia [1]. As of now, this devastating disease imposes its burden on over 55 million individuals globally. According to the most recent data from the World Health Organization (WHO), this figure is projected to escalate dramatically, with an estimated 139 million individuals anticipated to be living with dementia by the year 2050 [2].

AD is distinguished by a steady decline in cognitive abilities, which leads to the erosion of daily life skills and triggers behavioral disturbances as the disease progresses. From a neuropathological perspective, the brains of those suffering from AD exhibit significant atrophy. Additionally, two pathological anomalies are observed, namely extracellular amyloid plaques – predominantly made up of amyloid-beta peptides (Aβ) – and neurofibrillary tangles (NFTs). These NFTs are intracellular aggregates of hyperphosphorylated tau proteins (p-tau)~~, found~~ with phosphorylations occurring at multiple amino acid locations [3]. According to neuropathological studies, it is not plaque and tangle pathology but rather synapse loss that best correlates with cognitive symptoms [4] in AD, which is also apparent in early disease stages (maybe even before established plaque and tangle pathology)[3]. This is reflected by decreased levels of synaptic proteins in AD brain tissue [5]. ~~However, e~~Emerging research in recent years further supports the premise that significant disturbances in neural circuit connectivity, synaptic losses, and the decline in synaptic function often precede neuronal death and overt tau pathology [6]. Indeed, modifications in synaptic plasticity are increasingly being recognized as potential culprits behind the cognitive impairments witnessed in numerous brain diseases. Importantly, it is in the synaptic junctions where the destructive path of Aβ pathology may translate into tau alterations. In addition, growing research points towards alterations in the molecular components of synaptic plasticity pathways as the primary cause of these disorders. As such, synaptic transmission and plasticity serve as critical benchmarks in outlining the fundamental mechanisms of these synaptopathies. Furthermore, they provide a valuable platform for identifying potential therapeutic targets within preclinical models [7].

In recent years, a set of candidate biomarkers that signal synaptic dysfunction and loss have been identified from a combination of preclinical and clinical studies. These include neurogranin (Ng), synaptic vesicle glycoprotein 2A (SV2A), α- and β-synuclein, and visinin-like protein 1 (VILIP-1). The current challenge lies in drawing correlations between the progressive alterations in these biomarker levels and cognitive status in living patients. In addition to the classical biomarkers $A\beta_{1-42}$, t-tau, and p-tau measured in biological fluids or through neuroimaging studies, other biomarkers

have gained attention. Among these, a recent genome-wide analysis identified novel loci influencing plasma apolipoprotein E concentration and AD risk [8]. Also, neurophysiological markers of GABAergic, glutamatergic, and cholinergic neural circuits using specific transcranial magnetic stimulation (TMS) paradigms help to discriminate patients with MCI or AD versus healthy controls (HCs) [9]. Overall, combining information from different types of biomarkers will lead to earlier and more accurate diagnosis, improved monitoring, and better understanding of the complex mechanisms underlying AD. In this review, we offer a comprehensive overview of the fluid and PET biomarkers indicative of synaptic pathology at both pre-synaptic and post-synaptic levels, across the clinical *continuum* of AD (**Table 1**). We also explore the potential limitations and future implications of leveraging these promising biomarkers to enable early disease detection and track the impact of drug interventions in clinical trials.

IDENTIFYING EARLY MARKERS OF SYNAPTIC DYSFUNCTION: PRECLINICAL EVIDENCE

~~Animal models of NDs have primarily been instrumental in discerning the underlying mechanisms of neurodegeneration. Therefore, evaluating biomarkers from human blood (plasma) and cerebrospinal fluid (CSF) – derived from these animal models – holds considerable significance in assessing the pathophysiological mechanisms. In proof-of-concept studies, these biomarkers could also validate the efficacy of potential treatments for humans. Conversely, data gathered from patients can be effectively extrapolated and analyzed in animal models. This approach aids in understanding the predictive capacity and the pathophysiological implications of specific biomarkers. However, differences in disease mechanisms and lifespan between humans and animals pose certain limitations in comprehending the fundamental causes of NDs. While animal models that mimic the genetic characteristics of AD have been developed, they have only provided a limited amount of evidence on fluid biomarkers. For example, the Tg2576 AD model, which expresses the Swedish double mutation of the amyloid precursor protein (APP), demonstrated reduced CSF concentrations of the 42-amino acid-long Aβ peptide ($A\beta_{1-42}$). However, there was no significant change in the levels of the 40-amino acid-long Aβ peptide ($A\beta_{1-40}$) in aged Tg mice exhibiting Aβ pathology, when compared to their wild-type (WT) littermates[6]. An analogous trend in biomarkers was noted in human individuals affected by the disease[7]. Contrary to the findings in AD[12], PDAPP mice[9] exhibit elevated concentrations of $A\beta_{1-42}$. This increase has a positive correlation with the Aβ plaque load.~~

Upon thorough investigation, correlations often emerge between presynaptic and postsynaptic neuronal biomarkers found in patients' biofluids and brain pathology in AD animal models. The primary suspect for neuronal dysfunction in AD is likely the gradual disruption of synapses, and these

alterations can be tracked and analyzed in animal models at both cellular and subcellular levels. Early in the disease course, these changes can typically be attributed to functional or structural modifications at the presynaptic or postsynaptic sites and can result in memory failure [10–13].

Besides the classical cholinergic hypothesis of AD, recent data suggests that alteration of the catecholaminergic system plays a role in prodromal AD [14, 15]. In particular, the role of dopamine has been proved in a validated AD mouse model [16] and confirmed in AD patients by different imaging tools [17–19]. Hence, the catecholaminergic synapses constitute vulnerable functional elements during the prodromal phase of AD, likely contributing to the appearance of neuropsychiatric symptoms that appear in the early stages of AD [20]. Understanding the complex interplay between dopamine dysfunction and AD pathology remains an active and important area of research.

In AD, the production and deposition of Aβ proteins are common phenomena. Soluble Aβ oligomers (AβOs) have been found to be harmful to synapse function, leading to a decrease in synapse number. This effect may be a result of their interaction with postsynaptic membrane proteins, like prion, PirB, and EphB2, which can alter synaptic plasticity through changes in NMDA-type glutamate receptor and/or metabotropic glutamate receptor 5 (mGlu5) receptor[21]. Furthermore, AβOs can interact with trans-synaptic proteins like neurexins (Nrxs) and neuroligins (NLs), which are believed to mediate synapse damage, leading to memory loss in mice [22].

Glucose hypometabolism is frequently one of the earliest indicators of AD. Notably, in the cortex of young APP23 mice, energy metabolism appears to be significantly altered. This is evidenced by a marked increase in carbonylated proteins, which may well represent the earliest observable effect of amyloid precursor protein (APP) mutation or overexpression. This oxidative protein modification establishes a connection between energy metabolism and altered synaptic transmission [23]. Synapse morphology is another factor that could potentially influence the physiological synaptic activity. This is exemplified in hAPP-J20 mice, a transgenic model for early-onset AD. In these animals, the synaptic transmission and intrinsic excitability of the prominent h-current (PH) cells are diminished. Additionally, when compared to control mice, PH cells in hAPP-J20 mice demonstrated fewer dendritic intersections. This evidence supports the notion that abnormal synapse morphology may play a significant role in reducing neuronal activity [24].

Typically, synaptic loss or dysfunction is tracked primarily through biomarkers observed at the presynaptic level, with the exception of Ng. Among these, several proteins play crucial roles in the assembly of synaptic vesicles and the ensuing neurotransmitter release, including synaptotagmin-1 and the synaptosome associated protein 25 (SNAP-25). Specifically, SNAP-25 serves as an essential protein in synaptic vesicle exocytosis, making it a reliable biomarker of functional synapses. Notably, SNAP-25 levels have been found to be significantly diminished in the hippocampus of triple-

transgenic (3×Tg-AD) mice in comparison to non-Tg mice [25]. Another noteworthy biomarker is the growth-associated protein 43 (GAP-43), a presynaptic phosphoprotein discovered in the cerebrospinal fluid (CSF). GAP-43, primarily, contributes to terminal axon growth and differentiation and is implicated in synaptic remodeling that underpins learning and memory in adult animals. This is particularly evident in the consolidation of long-term memory [26].

Synucleins, like α-synuclein (α-syn), are functionally relevant proteins that exist in two forms: one is soluble and found in the cytoplasm, while the other attaches to membrane lipids, similar to what happens with synaptic vesicles. The significance of α-syn in AD is clearly illustrated in the doubly transgenic hSYN/hAPP mouse model. This model is typified by cognitive and motor changes, the loss of cholinergic neurons, synaptophysin-reactive presynaptic terminals with distinct signal intensity, extensive amyloid plaques, and hSYN-reactive intraneuronal fibrillar inclusions [27]. Furthermore, α-syn interacts with Aβ and tau to form toxic hetero-oligomers. Remarkably, reducing α-syn can thwart the degeneration of cholinergic neurons, thereby improving the associated deficits [28]. In fact, both α-syn and β-synuclein (β-syn), play integral roles in basal synaptic transmission, a fact evidenced by experiments involving double-knockout mice lacking either or both forms of synuclein [29]. Likely, the mechanisms by which they function are tied to changes in the release probability of neurotransmitters, a process characterized by synaptic vesicle mobilization or trafficking from the reserve pool to one that is readily releasable. Intriguingly, β-syn exhibits a difference from α-syn, in that it lacks the majority of the hydrophobic non-Aβ component found in the AD amyloid region [30]. Consequently, there is compelling evidence that suggests β-syn may serve a protective function against α-synucleinopathies, primarily because α-syn is prone to self-aggregation, leading to the formation of toxic protofibrils [31].

The development of a comprehensive panel of potential synaptic protein biomarkers is anticipated to provide an effective measure of various facets of synaptic loss, including pre-synaptic, synaptic vesicle, and dendritic. This panel could also serve as a reliable indicator of progressive memory decline. A recent study, for example, revealed reductions in the CSF concentration of several synaptic proteins, such as calsyntenin-1, GluR4, neurexin-2A, neurexin-3A, syntaxin-1B, and Thy-1 membrane glycoprotein, in the preclinical stages of AD, even before the detection of neurodegeneration biomarkers [32]. This unique protein signature may hold clinical value in tracking disease progression, especially during the preclinical stages of AD [32].

Ng and VILIP-1 are promising CSF biomarkers being investigated for their potential utility in the clinical diagnosis of AD. Ng, a post-synaptic protein, plays a crucial role in regulating synaptic plasticity. Specifically, it enhances synaptic transmission by modulating calmodulin (CaM) distribution within dendritic spines. Consequently, Ng overexpression in these spines triggers a

translocation of CaM towards the plasma membrane, subsequently reducing the induction threshold for long-term potentiation (LTP) [33, 34]. CaM is known to regulate a variety of enzymes, including the calcium/calmodulin dependent protein kinase II (CaMKII). In this context, studies on Ng knockout mice have demonstrated impaired CaMKII activation and LTP induction, thereby confirming the correlation between Ng expression and CaM-dependent enzymes [35]. Intriguingly, the expression of Ng in the hippocampus of mice has been found to be associated with performance in cognitive behavioral tasks; a decrease in Ng expression correlates with cognitive impairment [36]. Furthermore, the concentration of Ng has been linked with other biomarkers, such as VILIP-1. VILIP-1, a neuronal calcium-sensor protein predominantly expressed in neurons, plays a critical role in the regulation of excitotoxic neuronal damage and death, which fundamentally hinges on the disruption of Ca^{2+} homeostasis. Not only does VILIP-1 participate in various mechanisms that control synaptic plasticity and cognition, but it also significantly contributes to the pathophysiology of AD through the initiation of calcium-mediated neuronal death [37].

BIOMARKERS OF SYNAPSE DYSFUNCTION AND LOSS: CLINICAL EVIDENCE

Cognitive impairment, a paramount characteristic of AD, has been found to be closely linked with both synaptic dysfunction and loss. This has been evidenced by two distinct research approaches: (I) the quantification of synaptic protein concentrations [4], and (II) the estimation of synapse numbers using electron microscopy [38]. Both methodologies have unveiled a significant correlation between synaptic proteins/synapse numbers and cognitive scores, as gauged by the Mini-Mental State Examination (MMSE) scale [39]. Despite ongoing research, the impact of Aβ and tau pathologies on synaptic activity remains unclear [40]. However, studies have exposed soluble oligomeric forms of Aβ and tau as synaptotoxic species [41–43]. For example, Aβ oligomers have a negative impact on LTP, which is the fundamental molecular and cellular mechanism that forms and stores memories. This occurs possibly by interfering with the functionality of the N-methyl-D-aspartate (NMDA) receptors (NMDARs) and their downstream pathways [44]. Aβ oligomers also trigger oxidative stress, harm axonal transport, and induce neuronal death [45]. Oligomeric tau is present in pre- and postsynaptic terminals, and it has been suggested that accumulation of oligomeric tau in synapses is an early event in pathogenesis and that tau pathology may progress through the brain via trans-synaptic spread in human disease [46]. Indeed, phosphorylated tau oligomers may affect both NMDAR and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPARs). This results in a weakened glutamatergic transmission [47, 48].

AD is characterized by a gradual progression of synaptic dysfunction and loss [49]. In 1996, a seminal neuropathological study in 19 patients with AD and 9 age-matched control individuals found

that synaptic vesicle membrane protein rab3a levels were significantly reduced in the AD brain in a time and disease severity manner [5]. Later studies have documented in a consistent way the progression of synapse loss in the AD continuum, with approximately 15-20% loss in the neocortex and limbic system in MCI and early AD, which later increases to 20-50% loss [50]. Given the fundamental role synapses serve in cognition, significant efforts have been dedicated in identifying biomarkers of synaptic pathology that could provide insights into synaptic and cognitive functionality in AD. In 2019, a study involving two independent cohorts (the University of California San Diego [UCSD] and the Alzheimer's Disease Neuroimaging Initiative [ADNI] cohorts) found that synaptic biomarkers in CSF discriminated AD and controls and correlated strongly with cognition in AD and MCI and predicted cognitive decline beyond $A\beta_{1-42}$ and tau [51]. In 2021, a cross-sectional study in 397 participants has shown that CSF synaptic biomarkers increase in the early preclinical stages of the AD continuum even when a low burden of Aβ pathology is present [52]. From a clinical perspective, synaptic biomarkers help bridge the gap between synaptic degeneration and the patient's cognitive decline. They can be utilized alongside cognitive assessments to gain a more precise understanding of the patient's symptoms, especially in the early stages. Moreover, synaptic biomarkers offer a means to evaluate the impact of treatments on synaptic activity during drug trials[39, 40]. Broadly speaking, biomarkers indicating synaptic dysfunction have been identified in blood (plasma) or CSF. Depending upon the precise localization of the protein biomarker, these can be classified into two categories: (I) presynaptic or axonal biomarkers, and (II) postsynaptic or dendritic biomarkers (**Figures 1** and **2**).

Fluid Biomarkers of Synaptic Loss

Recent advancements in the realms of mass spectrometry and immunoassays have paved the way for the efficient quantification of synaptic protein markers in biofluids. These biomarkers include α-syn, VILIP-1, synaptotagmin-1, SNAP-25, GAP-43, which are found at the presynaptic level, and Ng, which is located at the postsynaptic level [39, 40].

Neurogranin. The protein Ng is predominantly localized within the dendritic spines of neurons in the hippocampus, amygdala, caudate, and putamen [53, 54]. When it interacts with the Ca^{2+}-binding protein CaM, it facilitates the modulation of Ca^{2+} signaling and synaptic plasticity by operating at a postsynaptic level [55]. As a result, this protein plays a crucial role in facilitating communication among signaling pathways associated with synaptic plasticity [56]. Originally, techniques such as immunoprecipitation and western blotting were used to reveal elevated concentrations of CSF Ng in AD [57]. The advent of immunoassay-based technologies has further confirmed this finding of increased CSF Ng levels in AD compared to controls [58–66]. Interestingly, this elevation was found

to be specifically associated with AD, excluding sporadic Creutzfeldt-Jakob disease (CJD) [67]. Studies have shown that higher concentrations of CSF Ng in prodromal AD are a significant predictor of faster progression to AD [58–60]. Furthermore, individuals with MCI who later progressed to AD (MCI-AD) tend to have higher CSF Ng levels than those whose conditions remained stable (stable-MCI) [59, 60]. Further investigations have found a correlation between increased CSF Ng levels and the severity of cognitive impairment, brain volume loss, and glucose hypometabolism in prodromal AD [60, 63]. In summary, CSF Ng stands out as the most well-established biomarker effectively mapping out synaptic loss or dysfunction associated with AD. It demonstrates a high degree of specificity for AD, making it an appropriate marker for detecting the initial stages of neuronal degeneration [40].

Utilizing both mass spectrometry technologies and traditional ELISA assays, it is possible to accurately measure the plasma concentrations of Ng. However, these plasma values remain unaltered in AD and do not exhibit any correlation with the corresponding CSF values. This lack of association is likely attributable to the impact of peripherally expressed Ng peptides – which possess the potential to skew the precision of blood Ng measurements [58].

Alpha-Synuclein. The protein α-syn is believed to play a vital role in the presynaptic regulation of cellular vesicle trafficking [68]. Lewy bodies are formed as a result of the accumulation of hyperphosphorylated forms of α-syn, which aggregate into insoluble fibrils within the brain [69]. These neuronal cytoplasmic inclusions are a hallmark of several brain proteinopathies with neurodegeneration, including Parkinson's disease (PD), PD with Dementia, and Lewy bodies dementia (LBD)[70]. Furthermore, the role of α-syn as a potential surrogate biomarker for synaptic loss in non-synucleinopathy NDs, including AD, is underscored by both its cellular localization and function. A notable increase in CSF α-syn concentrations was observed in AD patients, compared to ~~healthy controls (~~HCs~~)~~ and patients with other NDs [41, 71–73]. Additionally, positive correlations were established between CSF α-syn concentrations and cerebral Aβ load, as well as CSF concentrations of total tau (t-tau) and tau hyperphosphorylated at threonine 181 (p-tau$_{181}$) in asymptomatic individuals who are at risk of developing AD.

Consequently, a potential association may exist between the elevated levels of CSF α-syn and the early onset of AD, particularly in relation to both Aβ- and tau-related pathophysiological mechanisms during the asymptomatic phase of the disease [74]. However, these observations are frequently influenced by a multitude of technical factors. Additionally, α-syn is highly expressed outside the CNS, especially in blood, with red blood cells (RBCs) being its primary source. Therefore, the possibility of blood contamination during CSF collection implies a significant cause of variability, which subsequently casts doubt on the reliability of α-syn as a diagnostic biomarker [40]. Despite

these challenges, efforts have been made to evaluate α-syn as a potential blood biomarker for various dementias. One analysis revealed decreased serum α-syn levels in LBD compared to AD and HCs [75]. However, the same study found no significant difference when comparing AD patients with HCs. Additional research conducted on RBCs indicated lower α-syn values in PD and AD compared to HCs. Specifically, one study reported reduced levels of α-syn and its heterocomplexes with Aβ and tau (i.e., α-syn/Aβ and α-syn/tau) in the RBCs of AD patients compared to HCs. The study concluded that both RBC α-syn/Aβ and RBC α-syn/tau heterocomplexes could fairly differentiate AD patients from HCs [76]. In a subsequent study, it was discovered that RBC concentrations of α-syn and α-syn/tau heterocomplex were lower in LBD and AD compared to HCs, whereas RBC α-syn/$A\beta_{1-42}$ heterocomplex concentrations were only reduced in AD. Consequently, RBC α-syn heteromers could potentially serve as effective discriminators between NDs, including both LBD and AD, and HCs [77].

Beta-Synuclein. β-syn, a protein closely related to α-syn, is primarily located in the presynaptic region and plays a significant role in neuronal plasticity. It is also postulated to inhibit α-syn aggregation. This protein is notably prevalent in the NFTs found in AD. The first detection of elevated β-syn concentrations in CSF of AD patients was reported in 2016 [78]. Consequently, β-syn is progressively being recognized as a promising biomarker in blood for tracking synaptic degeneration in AD [79]. A recent study, involving 108 AD patients and individuals with other cognitive deficits, demonstrated increased β-syn concentrations in both blood and CSF in Aβ-PET positive ~~(Aβ+)~~ compared to Aβ-PET negative ~~(Aβ-)~~ participants [80]. Researchers also discovered a correlation between blood β-syn concentrations and Aβ-PET positivity across several brain regions. Interestingly, blood β-syn levels were found to predict Aβ status in individuals with MCI [80], although the mechanistic link between blood β-syn concentrations, Aβ deposition, and synaptic degeneration remains unclear.

A groundbreaking study examined β-syn levels in individuals with no cognitive impairment (i.e., preclinical AD), and those with MCI. They were categorized based on their Aβ-PET imaging status into Aβ-PET positive ~~(Aβ+)~~ and Aβ-PET negative ~~(Aβ-)~~ groups. The same categorization was applied to AD dementia patients, using either Aβ-PET imaging or the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio. The findings revealed increased plasma β-syn levels in preclinical AD, with even higher levels observed in those with MCI and AD dementia. Additionally, plasma β-syn concentrations were found to have significant associations with both Aβ and tau pathologies, as well as with temporal cortical atrophy and cognitive decline. This suggests that plasma β-syn could potentially serve as a biomarker for synaptic dysfunction, detectable from the disease's preclinical stages [81].

Visinin-like Protein 1. Dysregulation of Ca^{2+} homeostasis, commonly associated with AD pathophysiology, underscores the impact of neuronal Ca^{2+} alterations in NDs. These alterations are regulated by neuronal calcium sensor (NCS) proteins, among which VILIP-1 is noteworthy. VILIP-1, predominantly found in neurons, can influence the intraneuronal signaling pathways that govern synaptic plasticity. Crucially, it plays a significant role in the pathomechanisms of Ca^{2+} dysregulation, thereby contributing to neuronal loss [82]. Remarkably, a decrease in intraneuronal VILIP-1 expression has been observed in AD, primarily within the entorhinal cortex, when compared to control brains [83]. Interestingly, a significant correlation between the CSF concentrations of VILIP-1, p-tau, and t-tau has been reported, underscoring VILIP-1's potential as a biomarker for neuronal injury[84]. More importantly, CSF analyses revealed higher levels of VILIP-1 in AD patients compared to cognitively HCs [84, 85], despite one study presenting conflicting results[58]. Nevertheless, this same study indicated that baseline VILIP-1 values in patients with MCI successfully predicted their progression to AD [58]. Furthermore, the presence of this protein proved advantageous in distinguishing AD from other forms of dementia, such as LBD, frontotemporal lobar degeneration (FTLD), and progressive supranuclear palsy [86–88]. In addition, it demonstrated capabilities in predicting both global and regional (specifically, hippocampus and entorhinal cortex) [89] brain atrophy rates, as well as cognitive decline [86, 90]. As such, CSF VILIP-1 serves as a proxy marker for neurodegeneration in the early stages of AD, presenting significant diagnostic value. Longitudinal studies carried out on ~~subjects~~ participants with autosomal-dominant AD (ADAD) from the Dominantly Inherited Alzheimer Network (DIAN) Observational Study [91], and late-onset AD (LOAD) from the ~~Alzheimer's Disease Neuroimaging Initiative (~~ADNI~~)~~ study [92], underscored an initial increase in CSF VILIP-1 concentrations. This surge, however, is followed by a decrease as the disease advances, mirroring the trends observed with other neural injury biomarkers. A recent meta-analysis revealed that the average concentrations of CSF VILIP-1 were noticeably higher in AD patients than in cognitively HCs [93]. However, VILIP-1 levels did not effectively distinguish AD from MCI. Additionally, there were no significant differences between AD and LBD. Intriguingly, VILIP-1 was successful in significantly differentiating individuals with MCI-AD from those with stable-MCI. These findings underscore the potential utility of VILIP-1 as a biomarker that could aid in differentiating AD from controls, as well as between MCI-AD and stable-MCI individuals [93].

The available research conducted on VILIP-1 levels in blood plasma is somewhat limited, but a groundbreaking study revealed significantly elevated mean plasma VILIP-1 concentrations in patients displaying early symptoms of AD compared to cognitively HCs [86]. Although the differences in plasma VILIP-1 values between early AD patients and controls were significant, they were less pronounced than those observed in CSF. Despite this, these findings provide a promising

foundation for future studies focused on detecting VILIP-1 in blood and determining its diagnostic value [86].

Synaptotagmin-1. Synaptotagmin-1 is a membrane glycoprotein that resides within the synaptic vesicles and functions as a Ca^{2+} sensor. This protein plays a pivotal role in overseeing the neurotransmitter release in hippocampal neurons, which is heavily dependent on Ca^{2+} levels. Typically, the CSF concentrations of synaptotagmin-1 are found to be higher in both AD dementia and MCI-AD in comparison to cognitively HCs [94].

Synaptosomal-associated protein 25. SNAP-25 plays an integral role in the SNAP receptor (SNARE) complex, which is vital for the exocytotic release of neurotransmitters during synaptic transmission. This protein particularly assists in the fusion of synaptic vesicles with the presynaptic membrane and disruption of this process through genetic ablation of SNAP-25 can hinder synaptic transmission. Furthermore, SNAP-25 is potentially involved in modulating postsynaptic receptor trafficking, spine morphogenesis, and plasticity. These functions suggest that deficiencies in SNAP-25 could contribute to neurological diseases by impacting both presynaptic and postsynaptic activities [95]. Higher concentrations of CSF SNAP-25 have been observed in individuals with prodromal and AD dementia compared to HCs [96]. Both ELISA and mass spectrometry-based analyses have confirmed elevated CSF levels of SNAP-25 in AD patients, even in the very early stages of the disease [51, 96, 97]. A longitudinal study including patients with autosomal dominant AD found that CSF SNAP-25 concentrations were altered very early in the AD time course, approximately 15-19 years before estimated symptom onset and predicted performance on a cognitive composite scale, brain Aβ burden, hippocampal volume, and the estimated years from symptom onset [98]. However, another study involving AD and frontotemporal dementia (FTD) patients, as well as individuals with subjective cognitive decline (SCD) from the Amsterdam Dementia Cohort, found that CSF SNAP-25 concentrations may not be disease-specific since increased concentrations were observed both in AD and FTD when compared to SCD [99]. Notably, while SNAP-25 and Ng showed significant correlations with CSF t-tau and p-tau in all patient groups, neither correlated with CSF neurofilament light chain (NfL). This suggests that these synaptic biomarkers trace different pathological processes from axonal degeneration, which is monitored by NfL. Ultimately, these distinct synaptic biomarkers offer potential in shedding light on the pathological stages of various types of dementias.

Growth-Associated Protein 43. GAP-43 is called “plasticity” protein because it is expressed at high levels in neuronal growth cones during development and axonal regeneration [100]. GAP-43 is predominantly found in the hippocampus, entorhinal cortex, and neocortex regions of the adult brain, and its phosphorylated form plays a critical role in memory-related processes [101]. It was discovered

that CSF concentrations of this protein were significantly higher in AD patients compared to individuals showing no signs of NDs, who served as controls. Moreover, GAP-43 levels in AD patients surpassed those with other forms of NDs, suggesting that GAP-43 could serve as a specific biomarker for synaptic dysfunction associated with AD [102]. A cross-sectional study conducted in 384 middle-aged cognitively unimpaired participants at risk of developing AD has shown that CSF GAP-43 concentrations were significantly higher in Aβ-positive compared to Aβ-negative individuals. Interestingly, CSF GAP-43 concentrations were found to be significantly associated with higher brain metabolism but lower cortical thickness in AD-related brain regions [52]. To the best of our knowledge, there is no available data on blood concentrations of this protein.

Neuronal Pentraxin 2. Neuronal Pentraxin 2 (NPTX2) belongs to a unique family that includes neuronal pentraxins (NPTXs) and the neuronal pentraxin receptor (NPTXR). These components collectively form diverse NPTX complexes that engage with AMPA-type glutamate receptors, playing an integral role in various forms of synaptic plasticity during development and adulthood. Interestingly, NPTXs are found in both pre-synaptic and post-synaptic compartments, serving as potential indicators of both domains' status. This dual presence underscores the potential of NPTXs as markers for structural and functional synaptic disruptions, particularly in neurodegenerative conditions [103].

Studies have shown that both brain NPTX2 expression and its concentration in CSF are reduced in AD patients, and these reductions correlate with cognitive performance and the volume of the hippocampus. This evidence lends support to the idea that NPTX2 could serve as a valuable biomarker for AD [104]. A retrospective cross-sectional analysis has indicated lower CSF NPTX2 concentrations in Down syndrome, suggesting that CSF NPTX2 could be a promising surrogate marker for early AD-related changes in these individuals [105]. In a longitudinal study, it was revealed that lower CSF baseline NPTX2 concentrations were linked to earlier onset of MCI symptoms. Moreover, higher baseline concentrations of p-tau181 and t-tau were associated with greater initial NPTX2 values and faster rates of NPTX2 decline over time. Hence, NPTX2 could potentially serve as an effective prognostic biomarker during preclinical AD, enabling independent prediction of MCI onset in cognitively healthy individuals [106].

Positron Emission Tomography Biomarkers of Synaptic Loss

Regional Glucose Utilization. Regional brain glucose utilization is a well-established indicator of synaptic loss. In general, glucose hypometabolism is a reflection of the complex interplay between synaptic loss, neuronal cell death, and metabolic dysfunction [107]. The process of glucose utilization is also influenced by astroglial glutamate transport [108]. [^{18}F] fluorodeoxyglucose (FDG)-PET,

commonly referred to as [^{18}F]-FDG-PET, is extensively employed to monitor regional glucose uptake. AD patients typically exhibit a distinct pattern of reduced regional brain glucose utilization, prominently observed in the precuneus, posterior cingulate, parietal cortices, lateral temporal cortex, frontal cortices, and medial temporal lobe [109]. Notably, [^{18}F]-FDG-PET is also used extensively in assessing the preclinical, asymptomatic stage of AD.

Synaptic Vesicle Glycoprotein 2A. Synaptic vesicle glycoprotein 2A (SV2A) is a crucial component of the presynaptic vesicle membrane, characterized by its highly glycosylated nature. It displays a pervasive presence across all synaptic terminals, irrespective of their neurotransmitter content. The ubiquitous expression of SV2A in brain gray matter areas designates it as a promising candidate as a biomarker for synaptic density [110]. The non-invasive quantification of synaptic density, facilitated by this protein, is anticipated to enable early detection of NDs, potentially improving prognostic stratification [111]. PET tracers that target SV2A are gaining recognition as valuable biomarkers for identifying synaptic loss in AD. Various tracers have been designed, each characterized by their performance *in vivo*. Particularly, [^{11}C]UCB-J, a high-affinity ligand for SV2A, showed approximately 40% less binding to SV2A in the hippocampus region of AD patients compared with cognitively HCs [112]. However, the clinical utility of [^{11}C]UCB-J is limited due to the need for a cyclotron for its production. To overcome this, ^{18}F-labeled variants like [^{18}F]UCB-H have been introduced, although they exhibited a subpar specific signal in MCI individuals and AD patients compared to [^{11}C]UCB-J [113]. Interestingly, [^{18}F]MNI-1126 has shown promise as a potential PET tracer candidate in rats, yielding a signal analogous to [^{11}C]UCB-J [114]. Finally, initial testing of the racemate [^{18}F]MNI-1038 in non-human primates has yielded encouraging results [115].

DISCUSSION AND CONCLUSIONS

In general, except for Ng, ~~the majority of~~ most candidate biomarkers indicative of synaptic loss or dysfunction are detected at presynaptic level~~, with the exception of Ng~~. A variety of proteins, including synaptotagmin-1 and SNAP-25, are actively involved in the assembly of synaptic vesicles and the subsequent release of neurotransmitters. Notably, α-syn is present both as a soluble protein in the cytoplasm and in a form bound to membrane lipids, similar to the process seen with synaptic vesicles. **Figure 2** depicts in a schematic way the time course of three CSF synaptic biomarkers (Ng, VILIP-1, and SNAP-25) in relation to key CSF AD biomarkers. This is primarily based on a longitudinal analysis in autosomal dominant AD participants from the DIAN study [98] and in a cross-sectional study in middle-aged cognitively unimpaired participants at risk of developing AD [52]. The time-course of the alterations in CSF concentrations of Ng, VILIP-1, and SNAP-25 suggests

that synaptic damage begins just after brain Aβ accumulation and astrocytic activation, but earlier than neuroaxonal damage.

CSF synaptic biomarkers can improve the diagnosis of AD at an early stage as well as to monitor clinical progression. Current CSF synaptic biomarkers are altered in AD but seemingly not in other NDs [40]. This can reflect a higher response of synapses and neurons to Aβ-mediated damage, probably making AD the pathology with the highest synaptic damage. Among the reviewed synaptic proteins, Ng is the most extensively studied and the evidence presented thus far is seemingly specific for AD or Aβ deposition. On the other hand, several neuropathological studies have provided evidence that the AD brain is often affected by other pathologies other than amyloid plaques and NFTs. For many of these comorbid pathologies, the extent of synapse loss could be substantial [116]. These findings indicate that synapse loss is not a hallmark specific to AD but rather a change common to other neurological diseases. In addition, the possible contribution of peripheral expression of synaptic protein, as for example for Ng and α-synuclein, can represent a problem and can influence the validity of blood biomarkers of synaptic pathology.

Indeed, few studies have shown that synaptic biomarkers in blood may have promise in the clinic. Plasma levels of neuregulin 1 (NRG1), a growth and differentiation factor with a key role in the development and maintenance of synaptic transmission, were found to be increased in AD patients and correlated with CSF core AD and synaptic biomarkers and cognitive status [117]. However, a more concerted effort is needed to implement synaptic biomarkers in blood tests. Camporesi and colleagues ~~her his team~~ (2020) ~~has~~ proposed a systematic approach to this end. Initially, cerebral tissue studies could help unravel the pathophysiological mechanisms and suggest potential biomarkers. Subsequently, the focus should shift to exploring CSF, potentially succeeded by blood tests – a more cost-effective, less time-consuming, and easily accessible alternative to CSF. This stepwise approach could reveal a panel of synaptic biomarkers with clinical relevance [40]. One could ask what such markers would add to the existing biofluid-based and imaging biomarkers for amyloid, tau, and neurodegeneration pathologies in AD diagnostics. Since synapses are highly dynamic structures in the brain, it is possible that biomarkers for synaptic dysfunction or loss may normalize more rapidly in response to successful disease-modifying treatments. They may also be important to detect synaptic changes in a range of NDs, although the literature to date suggest that most of the synaptic biomarkers (except for NPTX2) show surprisingly AD-specific changes [40].

The potential of neuron-derived exosomes in blood, combined with the advent of highly sensitive, high-throughput, and investigative platforms such as mass spectrometry-based technologies typically used in proteomics, is anticipated to expedite the identification and quantification of synaptic proteins. For example, a study by Goetzl and colleagues (2018) revealed a decrease in the neuron-

derived exosome concentration of a synaptic protein signature – including synaptophysin, synaptopodin, synaptotagmin-2, and Ng – in the plasma of patients with AD and FTD compared to the control group [118]. In a separate study, the plasma concentration of other synaptic proteins derived from neural exosomes – encompassing NPTX2, neurexin 2, GRIA4, and neuroligin 1 – was found to be reduced in AD patients. Notably, the amount of GluR4 and neuroligin 1 were correlated with cognitive decline [119].

Intriguingly, an ongoing study is delving into the longitudinal relationships between alterations in Ng in plasma neural-derived exosomes and changes in hippocampal volume during the progression from amnestic MCI to AD [120]. Finally, a comprehensive meta-analysis, which examined the dynamics of both CSF and blood Ng concentrations in the AD clinical *continuum*, found elevated CSF Ng levels in AD compared to MCI, and in both these groups in comparison to HCs. Moreover, participants with MCI transitioning to AD exhibited higher CSF Ng values than those with stable MCI. An inverse correlation between cognitive decline and CSF Ng concentrations was also observed. Conversely, Ng levels in plasma exosomes from AD and MCI patients were lower compared to HCs, and levels in MCI-AD participants were further reduced compared to those in stable-MCI. This implies a potential clinical application of Ng, in both CSF and plasma exosomes, as a biomarker for cognitive status in AD and MCI-AD. However, additional analyses are needed to define the range of Ng values that can be used for diagnosis at different stages of the disease [121].

Despite the promising potential of CSF synaptic biomarkers, it is essential to acknowledge that most of these synaptic proteins do not possess the formal properties of AD biomarkers because their ability in predicting sporadic AD is unknown and some of them are not specific for AD [122, 123]. More studies with larger, more diverse cohorts and longer follow-up are needed to fully understand the clinical significance of some of synaptic proteins.

Another important point of attention is the fact that some synaptic biomarkers are widely expressed in the peripheral nervous system, mainly in the nerve and muscle junctions and in the autonomic ganglia; this fact represents a potential significant confounding factor when using synaptic biomarkers for AD prediction or monitoring. Finally, blood synaptic biomarkers, with very few exceptions, are at their infancy. These factors pose significant challenges to the wide use of synaptic biomarkers for diagnosis, biological monitoring, and pharmacological efficacy monitoring in routine clinical practice.

Synaptic loss is likely initiated by exposure to pathological Aβ and tau and represents a key mechanism of the AD cascade [50]. Thus, biomarkers of synaptic damage are potentially very useful to detect and interfere with the natural history of the disease. However, our knowledge of synapse proteome is still in its early phases. Synapses are highly diverse, and efforts are ongoing to identify

synapses that are affected at different stages of AD progression [50]. The molecular pathways enabling certain individuals to remain cognitively normal despite high levels of AD pathology largely remain a mystery of the AD biology. For instance, neuritin (NRN1), a neurotrophic factor previously linked to cognitive resilience, was shown to provide dendritic spine resilience against Aβ-induced neuronal hyperexcitability in cultured neurons [124]. Thus, the identification of synaptic proteins that make a neuron vulnerable or resilient during early or late stages of AD could represent a fundamental progress in the fight against AD.

Proteomic studies in brain, CSF or blood could lead to the identification of novel highly specific synaptic proteins that may help the understanding of how pathological synaptic processes evolve over the course of AD. Recently, this proteomic approach has been successfully executed by using CSF of patients with autosomal dominant AD [125]. The proteome allowed a better discrimination between mutation carriers and noncarriers than Aβ and tau measurements. The acquired information will be critical for developing precision therapeutic interventions and biomarkers for AD beyond those associated with Aβ and tau.

What does the future hold? Artificial intelligence (AI) and machine learning (ML) approaches are increasingly being used in dementia research and are emerging as promising tools for the early detection of AD [126] As previously discussed, neuroimaging techniques provide valuable insights into the structural and functional changes in the brain. AI and deep learning algorithms excel in analyzing large volumes of neuroimaging data and identifying patterns that may indicate the presence of AD. These algorithms can learn to recognize subtle changes in brain structure and connectivity that are often difficult for humans to detect [127].

AI algorithms can also integrate and analyze diverse biomarker data, including genetic markers, protein levels, and metabolic profiles, to identify specific patterns and signatures associated with AD pathology. Through machine learning, these algorithms can predict the likelihood of disease progression and assist in early diagnosis [128].

Electrophysiological recordings, such as electroencephalography (EEG) and magnetoencephalography (MEG), provide valuable insights into the electrical activity of the brain and synaptic functioning. AI and deep learning techniques can analyze these complex electrophysiological signals to identify aberrant synaptic activity patterns associated with synaptic diseases such as AD. By detecting subtle changes in synaptic transmission and neural oscillations, these algorithms might contribute to early detection and monitoring of disease progression [129].

In addition, AI models can analyze behavioral and cognitive data to detect patterns and markers associated with synaptic diseases. By leveraging machine learning algorithms, these models can identify subtle changes in behavior, attention, memory, and other cognitive domains that may indicate

early signs of synaptic dysfunction. This approach enables the objective and quantitative assessment of cognitive impairments, aiding in the early detection and monitoring of AD [130].

In conclusion, the use of AI and deep learning techniques for the early detection of AD or other synaptopathies holds great promise. While further research and validation are needed, the integration of AI in AD detection has the potential to improve diagnostic accuracy, enable early intervention, and facilitate the development of targeted therapies for this devastating condition.

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CONFLICT OF INTEREST

EE is the unique owner of 2E Science, a for-profit private scientific company. Neither **EE** nor 2E Science have any commercial interest or financial tie in relation with this article.

BPI is an employee at Chiesi Farmaceutici. He is listed among the inventors of a number of Chiesi Farmaceutici's patents of anti-Alzheimer drugs.

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

SL, **AS-L**, **NBM**, **AG**, **SL-O**, **JM-H**, **NM**, **CI**, **FC**, and **RN** declare that they have no conflicts of interest.

REFERENCES

1. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement. 2016;12:292–323.
2. World Alzheimer Report 2022 – Life after diagnosis: Navigating treatment, care and support.
3. Selkoe DJ. Alzheimer's Disease Is a Synaptic Failure. Science. 2002;298:789–791.
4. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, et al. Physical basis of cognitive alterations in alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Ann Neurol. 1991;30:572–580.
5. Blennow K, Bogdanovic N, Alafuzoff I, Ekman R, Davidsson P. Synaptic pathology in Alzheimer's disease: relation to severity of dementia, but not to senile plaques, neurofibrillary tangles, or the ApoE4 allele. J Neural Transm Vienna Austria 1996. 1996;103:603–618.
6. D'Amelio M, Nisticò R. Editorial Thematic Issue: Targeting Synaptic Dysfunction and Neural Connectivity in Neurological and Psychiatric Disorders. Curr Pharm Des. 2013;19:6391–6392.
7. Mango D, Saidi A, Cisale GY, Feligioni M, Corbo M, Nisticò R. Targeting Synaptic Plasticity in Experimental Models of Alzheimer's Disease. Front Pharmacol. 2019;10:778.
8. Aslam MM, Fan K-H, Lawrence E, Bedison MA, Snitz BE, DeKosky ST, et al. Genome-wide analysis identifies novel loci influencing plasma apolipoprotein E concentration and Alzheimer's disease risk. Mol Psychiatry. 2023. 5 September 2023. https://doi.org/10.1038/s41380-023-02170-4.
9. Mimura Y, Nishida H, Nakajima S, Tsugawa S, Morita S, Yoshida K, et al. Neurophysiological biomarkers using transcranial magnetic stimulation in Alzheimer's disease and mild cognitive impairment: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2021;121:47–59.
10. Mercerón-Martínez D, Ibaceta-González C, Salazar C, Almaguer-Melian W, Bergado-Rosado JA, Palacios AG. Alzheimer's Disease, Neural Plasticity, and Functional Recovery. J Alzheimers Dis. 2021;82:S37–S50.
11. Hefter D, Ludewig S, Draguhn A, Korte M. Amyloid, APP, and Electrical Activity of the Brain. The Neuroscientist. 2020;26:231–251.
12. Korte M, Herrmann U, Zhang X, Draguhn A. The role of APP and APLP for synaptic transmission, plasticity, and network function: lessons from genetic mouse models. Exp Brain Res. 2012;217:435–440.
13. Rowan MJ, Klyubin I, Cullen WK, Anwyl R. Synaptic plasticity in animal models of early Alzheimer's disease. Philos Trans R Soc Lond B Biol Sci. 2003;358:821–828.
14. Motta C, Assogna M, Bonomi CG, Di Lorenzo F, Nuccetelli M, Mercuri NB, et al. Interplay between the catecholaminergic enzymatic axis and neurodegeneration/neuroinflammation processes in the Alzheimer's disease continuum. Eur J Neurol. 2023;30:839–848.
15. Henjum K, Watne LO, Godang K, Halaas NB, Eldholm RS, Blennow K, et al. Cerebrospinal fluid catecholamines in Alzheimer's disease patients with and without biological disease. Transl Psychiatry. 2022;12:151.
16. Nobili A, Latagliata EC, Viscomi MT, Cavallucci V, Cutuli D, Giacovazzo G, et al. Dopamine neuronal loss contributes to memory and reward dysfunction in a model of Alzheimer's disease. Nat Commun. 2017;8:14727.
17. Sala A, Caminiti SP, Presotto L, Pilotto A, Liguori C, Chiaravalloti A, et al. In vivo human molecular neuroimaging of dopaminergic vulnerability along the Alzheimer's disease phases. Alzheimers Res Ther. 2021;13:187.
18. Serra L, D'Amelio M, Esposito S, Di Domenico C, Koch G, Marra C, et al. Ventral Tegmental Area Disconnection Contributes Two Years Early to Correctly Classify Patients

Converted to Alzheimer's Disease: Implications for Treatment. J Alzheimers Dis. 2021;82:985–1000.
19. Serra L, D'Amelio M, Di Domenico C, Dipasquale O, Marra C, Mercuri NB, et al. In vivo mapping of brainstem nuclei functional connectivity disruption in Alzheimer's disease. Neurobiol Aging. 2018;72:72–82.
20. Lyketsos CG, Carrillo MC, Ryan JM, Khachaturian AS, Trzepacz P, Amatniek J, et al. Neuropsychiatric symptoms in Alzheimer's disease. Alzheimers Dement. 2011;7:532–539.
21. Zhang H, Jiang X, Ma L, Wei W, Li Z, Chang S, et al. Role of Aβ in Alzheimer's-related synaptic dysfunction. Front Cell Dev Biol. 2022;10:964075.
22. Brito-Moreira J, Lourenco MV, Oliveira MM, Ribeiro FC, Ledo JH, Diniz LP, et al. Interaction of amyloid-β (Aβ) oligomers with neurexin 2α and neuroligin 1 mediates synapse damage and memory loss in mice. J Biol Chem. 2017;292:7327–7337.
23. Hartl D, Schuldt V, Forler S, Zabel C, Klose J, Rohe M. Presymptomatic Alterations in Energy Metabolism and Oxidative Stress in the APP23 Mouse Model of Alzheimer Disease. J Proteome Res. 2012;11:3295–3304.
24. Zhang X-Q, Xu L, Yang S-Y, Hu L-B, Dong F-Y, Sun B-G, et al. Reduced Synaptic Transmission and Intrinsic Excitability of a Subtype of Pyramidal Neurons in the Medial Prefrontal Cortex in a Mouse Model of Alzheimer's Disease. J Alzheimers Dis. 2021;84:129–140.
25. Cassano T, Serviddio G, Gaetani S, Romano A, Dipasquale P, Cianci S, et al. Glutamatergic alterations and mitochondrial impairment in a murine model of Alzheimer disease. Neurobiol Aging. 2012;33:1121.e1-1121.e12.
26. Maviel T, Durkin TP, Menzaghi F, Bontempi B. Sites of Neocortical Reorganization Critical for Remote Spatial Memory. Science. 2004;305:96–99.
27. Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, et al. β-Amyloid peptides enhance α-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci. 2001;98:12245–12250.
28. Spencer B, Desplats PA, Overk CR, Valera-Martin E, Rissman RA, Wu C, et al. Reducing Endogenous α-Synuclein Mitigates the Degeneration of Selective Neuronal Populations in an Alzheimer's Disease Transgenic Mouse Model. J Neurosci. 2016;36:7971–7984.
29. Chandra S, Fornai F, Kwon H-B, Yazdani U, Atasoy D, Liu X, et al. Double-knockout mice for α- and β-synucleins: Effect on synaptic functions. Proc Natl Acad Sci. 2004;101:14966–14971.
30. Liu S, Fa M, Ninan I, Trinchese F, Dauer W, Arancio O. ?-Synuclein involvement in hippocampal synaptic plasticity: role of NO, cGMP, cGK and CaMKII. Eur J Neurosci. 2007;25:3583–3596.
31. Fan Y, Limprasert P, Murray IVJ, Smith AC, Lee VM-Y, Trojanowski JQ, et al. β-synuclein modulates α-synuclein neurotoxicity by reducing α-synuclein protein expression. Hum Mol Genet. 2006;15:3002–3011.
32. Lleó A, Núñez-Llaves R, Alcolea D, Chiva C, Balateu-Paños D, Colom-Cadena M, et al. Changes in Synaptic Proteins Precede Neurodegeneration Markers in Preclinical Alzheimer's Disease Cerebrospinal Fluid. Mol Cell Proteomics. 2019;18:546–560.
33. Zhong L, Gerges NZ. Neurogranin Targets Calmodulin and Lowers the Threshold for the Induction of Long-Term Potentiation. PLoS ONE. 2012;7:e41275.
34. Petersen A, Gerges NZ. Neurogranin regulates CaM dynamics at dendritic spines. Sci Rep. 2015;5:11135.
35. Pak JH, Huang FL, Li J, Balschun D, Reymann KG, Chiang C, et al. Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: A study with knockout mice. Proc Natl Acad Sci. 2000;97:11232–11237.

36. Huang K-P, Huang FL, Jäger T, Li J, Reymann KG, Balschun D. Neurogranin/RC3 Enhances Long-Term Potentiation and Learning by Promoting Calcium-Mediated Signaling. J Neurosci. 2004;24:10660–10669.
37. Braunewell KH. The visinin-like proteins VILIP-1 and VILIP-3 in Alzheimer's disease—old wine in new bottles. Front Mol Neurosci. 2012;5.
38. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. Ann Neurol. 1990;27:457–464.
39. the Synaptic Health Endpoints Working Group, Colom-Cadena M, Spires-Jones T, Zetterberg H, Blennow K, Caggiano A, et al. The clinical promise of biomarkers of synapse damage or loss in Alzheimer's disease. Alzheimers Res Ther. 2020;12:21.
40. Camporesi E, Nilsson J, Brinkmalm A, Becker B, Ashton NJ, Blennow K, et al. Fluid Biomarkers for Synaptic Dysfunction and Loss. Biomark Insights. 2020;15:117727192095031.
41. Wang Z-Y, Han Z-M, Liu Q-F, Tang W, Ye K, Yao Y-Y. Use of CSF α-synuclein in the differential diagnosis between Alzheimer's disease and other neurodegenerative disorders. Int Psychogeriatr. 2015;27:1429–1438.
42. Tu S, Okamoto S, Lipton SA, Xu H. Oligomeric Aβ-induced synaptic dysfunction in Alzheimer's disease. Mol Neurodegener. 2014;9:48.
43. Guerrero-Muñoz MJ, Gerson J, Castillo-Carranza DL. Tau Oligomers: The Toxic Player at Synapses in Alzheimer's Disease. Front Cell Neurosci. 2015;9.
44. Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. Natural Oligomers of the Alzheimer Amyloid-β Protein Induce Reversible Synapse Loss by Modulating an NMDA-Type Glutamate Receptor-Dependent Signaling Pathway. J Neurosci. 2007;27:2866–2875.
45. Cline EN, Bicca MA, Viola KL, Klein WL. The Amyloid-β Oligomer Hypothesis: Beginning of the Third Decade. J Alzheimers Dis. 2018;64:S567–S610.
46. Colom-Cadena M, Davies C, Sirisi S, Lee J-E, Simzer EM, Tzioras M, et al. Synaptic oligomeric tau in Alzheimer's disease — A potential culprit in the spread of tau pathology through the brain. Neuron. 2023:S0896627323003057.
47. Puzzo D, Piacentini R, Fá M, Gulisano W, Li Puma DD, Staniszewski A, et al. LTP and memory impairment caused by extracellular Aβ and Tau oligomers is APP-dependent. eLife. 2017;6:e26991.
48. Jadhav S, Cubinkova V, Zimova I, Brezovakova V, Madari A, Cigankova V, et al. Tau-mediated synaptic damage in Alzheimer's disease. Transl Neurosci. 2015;6:214–226.
49. Cardozo PL, De Lima IBQ, Maciel EMA, Silva NC, Dobransky T, Ribeiro FM. Synaptic Elimination in Neurological Disorders. Curr Neuropharmacol. 2019;17:1071–1095.
50. Griffiths J, Grant SGN. Synapse pathology in Alzheimer's disease. Semin Cell Dev Biol. 2023;139:13–23.
51. Galasko D, Xiao M, Xu D, Smirnov D, Salmon DP, Dewit N, et al. Synaptic biomarkers in CSF aid in diagnosis, correlate with cognition and predict progression in MCI and Alzheimer's disease. Alzheimers Dement Transl Res Clin Interv. 2019;5:871–882.
52. Milà-Alomà M, Brinkmalm A, Ashton NJ, Kvartsberg H, Shekari M, Operto G, et al. CSF Synaptic Biomarkers in the Preclinical Stage of Alzheimer Disease and Their Association With MRI and PET: A Cross-sectional Study. Neurology. 2021;97:e2065–e2078.
53. Represa A, Deloulme J, Sensenbrenner M, Ben-Ari Y, Baudier J. Neurogranin: immunocytochemical localization of a brain-specific protein kinase C substrate. J Neurosci. 1990;10:3782–3792.
54. Gerendasy DD, Gregor Sutcliffe J. RC3/neurogranin, a postsynaptic calpacitin for setting the response threshold to calcium influxes. Mol Neurobiol. 1997;15:131–163.
55. Xia Z, Storm DR. The role of calmodulin as a signal integrator for synaptic plasticity. Nat Rev Neurosci. 2005;6:267–276.

56. Hayashi Y. Long-term potentiation: two pathways meet at neurogranin. EMBO J. 2009;28:2859–2860.
57. Thorsell A, Bjerke M, Gobom J, Brunhage E, Vanmechelen E, Andreasen N, et al. Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. Brain Res. 2010;1362:13–22.
58. Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Öhrfelt A, Andersson K, et al. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. Alzheimers Dement. 2015;11:1180–1190.
59. Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JackH, Scheltens P, et al. Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. JAMA Neurol. 2015;72:1275.
60. Portelius E, Zetterberg H, Skillbäck T, Törnqvist U, Andreasson U, Trojanowski JQ, et al. Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. Brain. 2015;138:3373–3385.
61. Kvartsberg H, Portelius E, Andreasson U, Brinkmalm G, Hellwig K, Lelental N, et al. Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls. Alzheimers Res Ther. 2015;7:40.
62. De Vos A, Jacobs D, Struyfs H, Fransen E, Andersson K, Portelius E, et al. C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. Alzheimers Dement. 2015;11:1461–1469.
63. Tarawneh R, D'Angelo G, Crimmins D, Herries E, Griest T, Fagan AM, et al. Diagnostic and Prognostic Utility of the Synaptic Marker Neurogranin in Alzheimer Disease. JAMA Neurol. 2016;73:561.
64. Sanfilippo C, Forlenza O, Zetterberg H, Blennow K. Increased neurogranin concentrations in cerebrospinal fluid of Alzheimer's disease and in mild cognitive impairment due to AD. J Neural Transm. 2016;123:1443–1447.
65. for the Alzheimer Precision Medicine Initiative (APMI), Lista S, Toschi N, Baldacci F, Zetterberg H, Blennow K, et al. Cerebrospinal Fluid Neurogranin as a Biomarker of Neurodegenerative Diseases: A Cross-Sectional Study. J Alzheimers Dis. 2017;59:1327–1334.
66. Sutphen CL, McCue L, Herries EM, Xiong C, Ladenson JH, Holtzman DM, et al. Longitudinal decreases in multiple cerebrospinal fluid biomarkers of neuronal injury in symptomatic late onset Alzheimer's disease. Alzheimers Dement. 2018;14:869–879.
67. Blennow K, Diaz-Lucena D, Zetterberg H, Villar-Pique A, Karch A, Vidal E, et al. CSF neurogranin as a neuronal damage marker in CJD: a comparative study with AD. J Neurol Neurosurg Psychiatry. 2019;90:846–853.
68. Wong YC, Krainc D. α-synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies. Nat Med. 2017;23:1–13.
69. Colom-Cadena M, Pegueroles J, Herrmann AG, Henstridge CM, Muñoz L, Querol-Vilaseca M, et al. Synaptic phosphorylated α-synuclein in dementia with Lewy bodies. Brain. 2017;140:3204–3214.
70. Goedert M. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein. Science. 2015;349:1255555.
71. Slaets S, Vanmechelen E, Le Bastard N, Decraemer H, Vandijck M, Martin J, et al. Increased CSF α-synuclein levels in Alzheimer's disease: Correlation with tau levels. Alzheimers Dement. 2014;10.
72. Hansson O, Hall S, Öhrfelt A, Zetterberg H, Blennow K, Minthon L, et al. Levels of cerebrospinal fluid α-synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease. Alzheimers Res Ther. 2014;6:25.

73. Mattsson N, Insel P, Tosun D, Zhang J, Jack Jr CR, Galasko D, et al. Effects of Baseline CSF α-Synuclein on Regional Brain Atrophy Rates in Healthy Elders, Mild Cognitive Impairment and Alzheimer's Disease. PLoS ONE. 2013;8:e85443.
74. Vergallo A, Bun R, Toschi N, Baldacci F, Zetterberg H, Blennow K, et al. Association of cerebrospinal fluid α-synuclein with total and phospho-tau $_{181}$ protein concentrations and brain amyloid load in cognitively normal subjective memory complainers stratified by Alzheimer's disease biomarkers. Alzheimers Dement. 2018;14:1623–1631.
75. Laske C, Fallgatter AJ, Stransky E, Hagen K, Berg D, Maetzler W. Decreased α-Synuclein Serum Levels in Patients with Lewy Body Dementia Compared to Alzheimer's Disease Patients and Control Subjects. Dement Geriatr Cogn Disord. 2011;31:413–416.
76. Baldacci F, Daniele S, Piccarducci R, Giampietri L, Pietrobono D, Giorgi FS, et al. Potential Diagnostic Value of Red Blood Cells α-Synuclein Heteroaggregates in Alzheimer's Disease. Mol Neurobiol. 2019;56:6451–6459.
77. Daniele S, Baldacci F, Piccarducci R, Palermo G, Giampietri L, Manca ML, et al. α-Synuclein Heteromers in Red Blood Cells of Alzheimer's Disease and Lewy Body Dementia Patients. J Alzheimers Dis. 2021;80:885–893.
78. Oeckl P, Metzger F, Nagl M, Von Arnim CAF, Halbgebauer S, Steinacker P, et al. Alpha-, Beta-, and Gamma-synuclein Quantification in Cerebrospinal Fluid by Multiple Reaction Monitoring Reveals Increased Concentrations in Alzheimer′s and Creutzfeldt-Jakob Disease but No Alteration in Synucleinopathies. Mol Cell Proteomics. 2016;15:3126–3138.
79. Mohaupt P, Pons M-L, Vialaret J, Delaby C, Hirtz C, Lehmann S. β-Synuclein as a candidate blood biomarker for synaptic degeneration in Alzheimer's disease. Alzheimers Res Ther. 2022;14:179.
80. Oeckl P, Bluma M, Bucci M, Halbgebauer S, Chiotis K, Sandebring-Matton A, et al. Blood β-synuclein is related to amyloid PET positivity in memory clinic patients. Alzheimers Dement. 2023:alz.13046.
81. Oeckl P, Janelidze S, Halbgebauer S, Stomrud E, Palmqvist S, Otto M, et al. Higher plasma β-synuclein indicates early synaptic degeneration in Alzheimer's disease. Alzheimers Dement. 2023:alz.13103.
82. Groblewska M, Muszyński P, Wojtulewska-Supron A, Kulczyńska-Przybik A, Mroczko B. The Role of Visinin-Like Protein-1 in the Pathophysiology of Alzheimer's Disease. J Alzheimers Dis. 2015;47:17–32.
83. Braunewell K-H, Riederer P, Spilker C, Gundelfinger ED, Bogerts B, Bernstein H-G. Abnormal Localization of Two Neuronal Calcium Sensor Proteins, Visinin-Like Proteins (VILIPs)-1 and -3, in Neocortical Brain Areas of Alzheimer Disease Patients. Dement Geriatr Cogn Disord. 2001;12:110–116.
84. Lee J-M, Blennow K, Andreasen N, Laterza O, Modur V, Olander J, et al. The Brain Injury Biomarker VLP-1 Is Increased in the Cerebrospinal Fluid of Alzheimer Disease Patients. Clin Chem. 2008;54:1617–1623.
85. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016;15:673–684.
86. Tarawneh R, D'Angelo G, Macy E, Xiong C, Carter D, Cairns NJ, et al. Visinin-like protein-1: Diagnostic and prognostic biomarker in Alzheimer disease. Ann Neurol. 2011;70:274–285.
87. Luo X, Hou L, Shi H, Zhong X, Zhang Y, Zheng D, et al. CSF levels of the neuronal injury biomarker visinin-like protein-1 in Alzheimer's disease and dementia with Lewy bodies. J Neurochem. 2013;127:681–690.
88. Babić Leko M, Borovečki F, Dejanović N, Hof PR, Šimić G. Predictive Value of Cerebrospinal Fluid Visinin-Like Protein-1 Levels for Alzheimer's Disease Early Detection

and Differential Diagnosis in Patients with Mild Cognitive Impairment. J Alzheimers Dis. 2016;50:765–778.
89. Tarawneh R, Head D, Allison S, Buckles V, Fagan AM, Ladenson JH, et al. Cerebrospinal Fluid Markers of Neurodegeneration and Rates of Brain Atrophy in Early Alzheimer Disease. JAMA Neurol. 2015;72:656.
90. Tarawneh R, Lee J-M, Ladenson JH, Morris JC, Holtzman DM. CSF VILIP-1 predicts rates of cognitive decline in early Alzheimer disease. Neurology. 2012;78:709–719.
91. Fagan AM, Xiong C, Jasielec MS, Bateman RJ, Goate AM, Benzinger TLS, et al. Longitudinal Change in CSF Biomarkers in Autosomal-Dominant Alzheimer's Disease. Sci Transl Med. 2014;6.
92. Sutphen CL, McCue L, Herries EM, Xiong C, Ladenson JH, Holtzman DM, et al. Longitudinal decreases in multiple cerebrospinal fluid biomarkers of neuronal injury in symptomatic late onset Alzheimer's disease. Alzheimers Dement. 2018;14:869–879.
93. Mavroudis IA, Petridis F, Chatzikonstantinou S, Karantali E, Kazis D. A meta-analysis on the levels of VILIP-1 in the CSF of Alzheimer's disease compared to normal controls and other neurodegenerative conditions. Aging Clin Exp Res. 2021;33:265–272.
94. Öhrfelt A, Brinkmalm A, Dumurgier J, Brinkmalm G, Hansson O, Zetterberg H, et al. The pre-synaptic vesicle protein synaptotagmin is a novel biomarker for Alzheimer's disease. Alzheimers Res Ther. 2016;8:41.
95. Antonucci F, Corradini I, Fossati G, Tomasoni R, Menna E, Matteoli M. SNAP-25, a Known Presynaptic Protein with Emerging Postsynaptic Functions. Front Synaptic Neurosci. 2016;8.
96. Brinkmalm A, Brinkmalm G, Honer WG, Frölich L, Hausner L, Minthon L, et al. SNAP-25 is a promising novel cerebrospinal fluid biomarker for synapse degeneration in Alzheimer's disease. Mol Neurodegener. 2014;9:53.
97. the Alzheimer's Disease Neuroimaging Initiative, Zhang H, Therriault J, Kang MS, Ng KP, Pascoal TA, et al. Cerebrospinal fluid synaptosomal-associated protein 25 is a key player in synaptic degeneration in mild cognitive impairment and Alzheimer's disease. Alzheimers Res Ther. 2018;10:80.
98. Schindler SE, Li Y, Todd KW, Herries EM, Henson RL, Gray JD, et al. Emerging cerebrospinal fluid biomarkers in autosomal dominant Alzheimer's disease. Alzheimers Dement. 2019;15:655–665.
99. Das S, Goossens J, Jacobs D, Dewit N, Pijnenburg YAL, In 'T Veld SGJG, et al. Synaptic biomarkers in the cerebrospinal fluid associate differentially with classical neuronal biomarkers in patients with Alzheimer's disease and frontotemporal dementia. Alzheimers Res Ther. 2023;15:62.
100. Rosskothen-Kuhl N, Illing R-B. Gap43 Transcription Modulation in the Adult Brain Depends on Sensory Activity and Synaptic Cooperation. PLoS ONE. 2014;9:e92624.
101. Benowitz LI, Routtenberg A. GAP-43: an intrinsic determinant of neuronal development and plasticity. Trends Neurosci. 1997;20:84–91.
102. Sandelius Å, Portelius E, Källén Å, Zetterberg H, Rot U, Olsson B, et al. Elevated CSF GAP-43 is Alzheimer's disease specific and associated with tau and amyloid pathology. Alzheimers Dement. 2019;15:55–64.
103. Gómez De San José N, Massa F, Halbgebauer S, Oeckl P, Steinacker P, Otto M. Neuronal pentraxins as biomarkers of synaptic activity: from physiological functions to pathological changes in neurodegeneration. J Neural Transm. 2022;129:207–230.
104. Xiao M-F, Xu D, Craig MT, Pelkey KA, Chien C-C, Shi Y, et al. NPTX2 and cognitive dysfunction in Alzheimer's Disease. eLife. 2017;6:e23798.
105. Belbin O, Xiao M-F, Xu D, Carmona-Iragui M, Pegueroles J, Benejam B, et al. Cerebrospinal fluid profile of NPTX2 supports role of Alzheimer's disease-related inhibitory circuit dysfunction in adults with Down syndrome. Mol Neurodegener. 2020;15:46.

106. Soldan A, Oh S, Ryu T, Pettigrew C, Zhu Y, Moghekar A, et al. NPTX2 in cerebrospinal fluid predicts the progression from normal cognition to Mild Cognitive Impairment. Ann Neurol. 2023:ana.26725.
107. Reiman EM. Fluorodeoxyglucose positron emission tomography: emerging roles in the evaluation of putative Alzheimer's disease-modifying treatments. Neurobiol Aging. 2011;32:S44–S47.
108. Zimmer ER, Parent MJ, Souza DG, Leuzy A, Lecrux C, Kim H-I, et al. [18F]FDG PET signal is driven by astroglial glutamate transport. Nat Neurosci. 2017;20:393–395.
109. Alexander GE, Chen K, Pietrini P, Rapoport SI, Reiman EM. Longitudinal PET Evaluation of Cerebral Metabolic Decline in Dementia: A Potential Outcome Measure in Alzheimer's Disease Treatment Studies. Am J Psychiatry. 2002;159:738–745.
110. Rossi R, Arjmand S, Bærentzen SL, Gjedde A, Landau AM. Synaptic Vesicle Glycoprotein 2A: Features and Functions. Front Neurosci. 2022;16:864514.
111. Cawthorne C, Maguire P, Mercier J, Sciberras D, Serdons K, Bormans G, et al. Human biodistribution and dosimetry of [11C]-UCB-J, a PET radiotracer for imaging synaptic density. EJNMMI Phys. 2021;8:37.
112. Chen M-K, Mecca AP, Naganawa M, Finnema SJ, Toyonaga T, Lin S-F, et al. Assessing Synaptic Density in Alzheimer Disease With Synaptic Vesicle Glycoprotein 2A Positron Emission Tomographic Imaging. JAMA Neurol. 2018;75:1215–1224.
113. Bastin C, Bahri MA, Meyer F, Manard M, Delhaye E, Plenevaux A, et al. In vivo imaging of synaptic loss in Alzheimer's disease with [18F]UCB-H positron emission tomography. Eur J Nucl Med Mol Imaging. 2020;47:390–402.
114. Patel S, Knight A, Krause S, Teceno T, Tresse C, Li S, et al. Preclinical In Vitro and In Vivo Characterization of Synaptic Vesicle 2A–Targeting Compounds Amenable to F-18 Labeling as Potential PET Radioligands for Imaging of Synapse Integrity. Mol Imaging Biol. 2020;22:832–841.
115. Constantinescu CC, Tresse C, Zheng M, Gouasmat A, Carroll VM, Mistico L, et al. Development and In Vivo Preclinical Imaging of Fluorine-18-Labeled Synaptic Vesicle Protein 2A (SV2A) PET Tracers. Mol Imaging Biol. 2019;21:509–518.
116. Scheff SW, Neltner JH, Nelson PT. Is synaptic loss a unique hallmark of Alzheimer's disease? Biochem Pharmacol. 2014;88:517–528.
117. Vrillon A, Mouton-Liger F, Martinet M, Cognat E, Hourregue C, Dumurgier J, et al. Plasma neuregulin 1 as a synaptic biomarker in Alzheimer's disease: a discovery cohort study. Alzheimers Res Ther. 2022;14:71.
118. Goetzl EJ, Kapogiannis D, Schwartz JB, Lobach IV, Goetzl L, Abner EL, et al. Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease. FASEB J. 2016;30:4141–4148.
119. Goetzl EJ, Abner EL, Jicha GA, Kapogiannis D, Schwartz JB. Declining levels of functionally specialized synaptic proteins in plasma neuronal exosomes with progression of Alzheimer's disease. FASEB J. 2018;32:888–893.
120. He M, Sun L, Cao W, Yin C, Sun W, Liu P, et al. Association between plasma exosome neurogranin and brain structure in patients with Alzheimer's disease: a protocol study. BMJ Open. 2020;10:e036990.
121. Liu W, Lin H, He X, Chen L, Dai Y, Jia W, et al. Neurogranin as a cognitive biomarker in cerebrospinal fluid and blood exosomes for Alzheimer's disease and mild cognitive impairment. Transl Psychiatry. 2020;10:125.
122. Nilsson J, Constantinescu J, Nellgård B, Jakobsson P, Brum WS, Gobom J, et al. CEREBROSPINAL FLUID Biomarkers of Synaptic Dysfunction are Altered in Parkinson's Disease and Related Disorders. Mov Disord. 2023;38:267–277.

123. Sogorb-Esteve A, Nilsson J, Swift IJ, Heller C, Bocchetta M, Russell LL, et al. Differential impairment of cerebrospinal fluid synaptic biomarkers in the genetic forms of frontotemporal dementia. Alzheimers Res Ther. 2022;14:118.
124. Hurst C, Pugh DA, Abreha MH, Duong DM, Dammer EB, Bennett DA, et al. Integrated Proteomics to Understand the Role of Neuritin (NRN1) as a Mediator of Cognitive Resilience to Alzheimer's Disease. Mol Cell Proteomics. 2023;22:100542.
125. Johnson ECB, Bian S, Haque RU, Carter EK, Watson CM, Gordon BA, et al. Cerebrospinal fluid proteomics define the natural history of autosomal dominant Alzheimer's disease. Nat Med. 2023;29:1979–1988.
126. Vrahatis AG, Skolariki K, Krokidis MG, Lazaros K, Exarchos TP, Vlamos P. Revolutionizing the Early Detection of Alzheimer's Disease through Non-Invasive Biomarkers: The Role of Artificial Intelligence and Deep Learning. Sensors. 2023;23:4184.
127. Borchert RJ, Azevedo T, Badhwar A, Bernal J, Betts M, Bruffaerts R, et al. Artificial intelligence for diagnostic and prognostic neuroimaging in dementia: A systematic review. Alzheimers Dement. 2023:alz.13412.
128. Winchester LM, Harshfield EL, Shi L, Badhwar A, Khleifat AA, Clarke N, et al. Artificial intelligence for biomarker discovery in Alzheimer's disease and dementia. Alzheimers Dement. 2023:alz.13390.
129. Rossini PM, Miraglia F, Alù F, Cotelli M, Ferreri F, Iorio RD, et al. Neurophysiological Hallmarks of Neurodegenerative Cognitive Decline: The Study of Brain Connectivity as A Biomarker of Early Dementia. J Pers Med. 2020;10:34.
130. Mandal PK, Shukla D. Brain Metabolic, Structural, and Behavioral Pattern Learning for Early Predictive Diagnosis of Alzheimer's Disease. J Alzheimers Dis. 2018;63:935–939.

FIGURES CAPTIONS

Figure 1. Comprehensive overview of pathological pathways and biomarkers indicative of synaptic damage in AD brain.

The process of synaptic degeneration in AD is driven by intricate interplays among factors such as Aβ and tau toxicity, calcium dysregulation, mitochondrial dysfunction, and changes in glial cells. A variety of synaptic proteins, sourced from both pre-synaptic and post-synaptic terminals, have been put forward as potential biomarkers for synaptic damage. In the pre-synaptic compartment, aberrantly folded tau proteins contribute to the loss of synaptic vesicle proteins (*e.g.,* synaptotagmin-1), and the depletion of the synaptic vesicle pool. Alpha-synuclein (α-syn) monomers and oligomers may inhibit vesicle fusion triggered by SNARE proteins and facilitate the phosphorylation of tau proteins. Furthermore, oligomeric Aβ can promote calcium influx, instigating excitotoxicity in both pre- and post-synaptic terminals. As for the post-synaptic terminal, Aβ has an affinity for metabotropic glutamate receptor 5 (mGluR5), leading to increased phosphorylation of synaptic tau and a decrease in NMDARs. These alterations in NMDARs correlate with enhanced LTD and diminished LTP, factors that facilitate synaptic loss. Calcium binding to CaM permits the phosphorylation of the AMPAR, which plays a significant role in LTP. NPTX2 can form a complex with NPTX1 at the post-synaptic membrane, leading to the aggregation of AMPARs. Ng plays a critical role in synaptic plasticity, synaptic regeneration, and LTP through the calcium and CaM signaling pathways. VILIP-1 is implicated in the pathological mechanisms of altered calcium homeostasis that result in neuronal loss. GAP-43 is an essential component of the axon and pre-synaptic terminal, which gets phosphorylated following LTP. SV2A is a synaptic vesicle protein that regulates neurotransmitter release.

Abbreviations: α-syn, alpha-synuclein; Aβ, amyloid beta; AD, Alzheimer's disease; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; APP, amyloid precursor protein; CaM, calmodulin; GAP-43, growth-associated protein-43; LTD, long-term depression; LTP, long- term potentiation; mGluR5, metabotropic glutamate receptor 5; Ng, neurogranin; NMDARs, N-methyl-D-aspartate receptors; NPTX2, neuronal pentraxin 2; SNARE, Soluble N-ethylmaleimide-Sensitive Factor Attachment Proteins Receptors; SV2A, synaptic vesicle protein 2A; VILIP-1, visinin-like protein 1.

Figure 2. Schematic representation of possible time-course of synaptic biomarkers in relation to key AD biomarkers.

This Figure is mainly based on a longitudinal study in autosomal dominant AD participants [98] and in a cross-sectional study in middle-aged cognitively unimpaired participants at risk of developing AD [52]. The time-course of changes in CSF concentrations of Ng, VILIP-1, and SNAP-25 suggests that synaptic damage begins just after brain Aβ accumulation and astrocytic activation, but earlier than neuroaxonal damage.

Abbreviations: $A\beta_{1-42}$, 42-amino acid-long Aβ peptide; $A\beta_{1-40}$, 40-amino acid-long Aβ peptide; $p\text{-}tau_{217}$, tau hyperphosphorylated at threonine 217; GFAP, glial fibrillary acid protein; NfL, neurofilament light chain; CSF, cerebrospinal fluid; Ng, neurogranin; SNAP-25, synaptosomal-associated protein, 25kDa; VILIP-1, visinin-like protein 1.

TABLES

Table 1. Summary of potential synaptic biomarkers explored within the clinical *continuum* of AD.

Pathophysiological pathway	Biomarker	Biological matrix	Direction (increase or decrease) of the biomarker concentration change
Synaptic pathology (synaptic dysfunction and loss)	Synaptotagmin-1	CSF	Increased in MCI and AD
	SNAP-25	CSF	Increased in prodromal AD and AD dementia
	GAP-43	CSF	Increased in AD
	α-syn	CSF	Increased in AD
	α-syn/Aβ and α-syn/tau heterodimers	Red blood cells	Decreased in AD
	β-syn	Plasma	Increased in MCI and AD
	Ng	CSF	Increased in MCI-AD, prodromal AD, and AD
		Plasma	No major changes in AD
		Plasma exosomes	Decreased in MCI-AD and AD
	NPTX2	CSF	Decreased in MCI, AD, and Down syndrome
Altered Ca^{2+} homeostasis	VILIP-1	CSF	Increased in MCI-AD and AD
		Plasma	Increased in AD

Abbreviations: α-syn, alpha-synuclein; Aβ, amyloid beta; AD, Alzheimer's disease; β-syn, beta-synuclein; CSF, cerebrospinal fluid; GAP-43, growth-associated protein-43; MCI, mild cognitive impairment; MCI-AD, mild cognitive impairment due to Alzheimer's disease; Ng, neurogranin; NPTX2, neuronal pentraxin 2; SNAP-25, synaptosomal-associated protein, 25 kDa; VILIP-1, visinin-like protein 1.

FIGURE 1

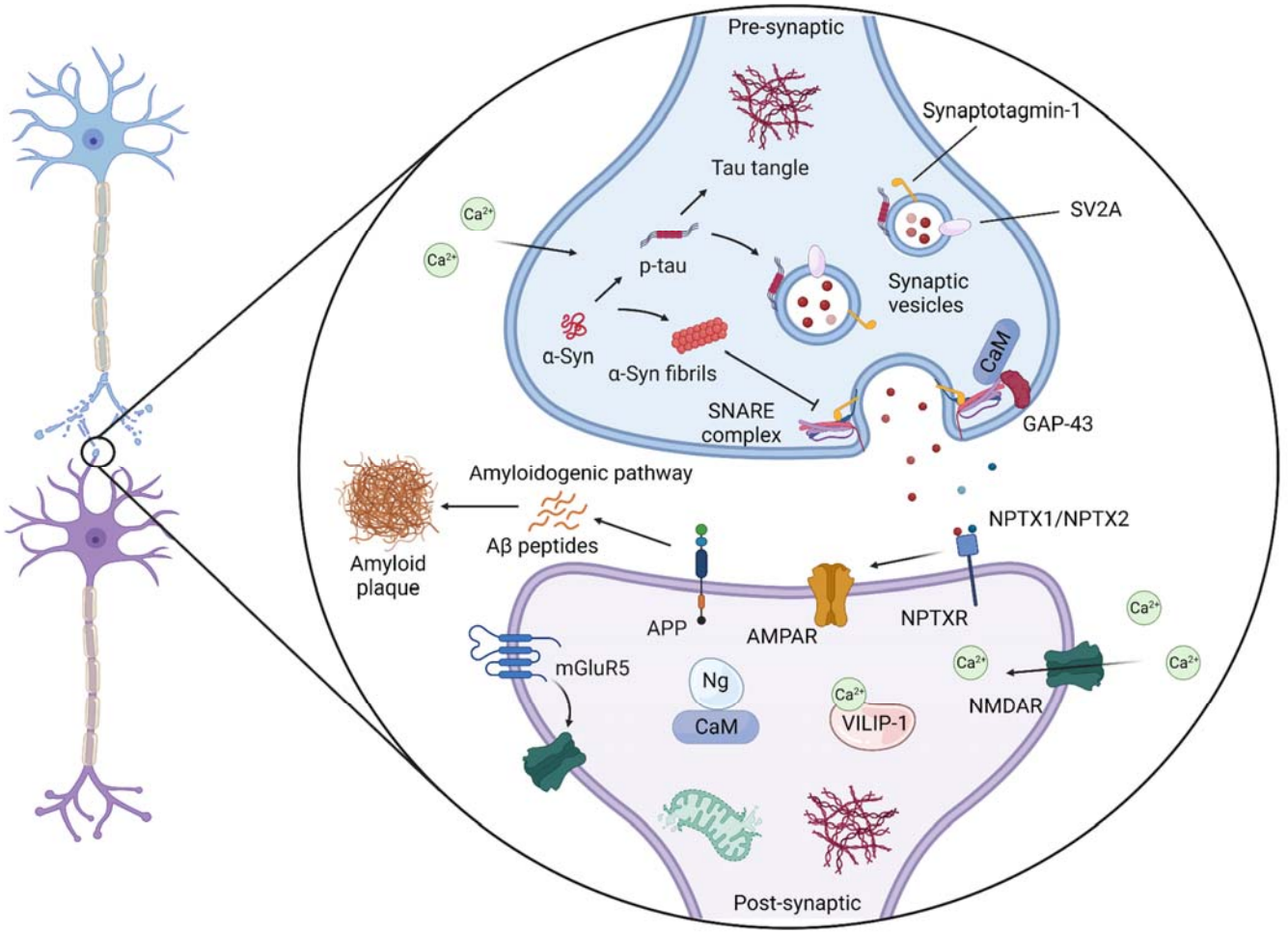
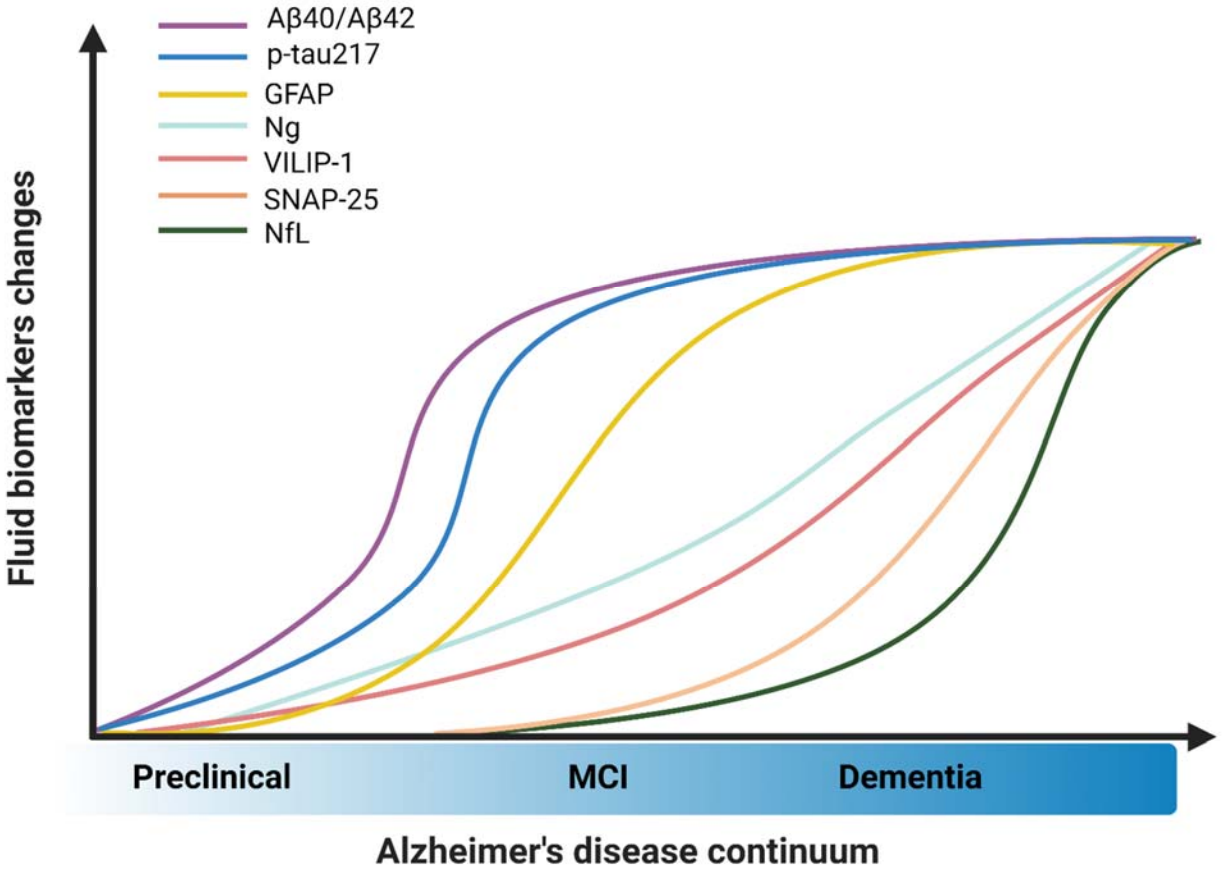
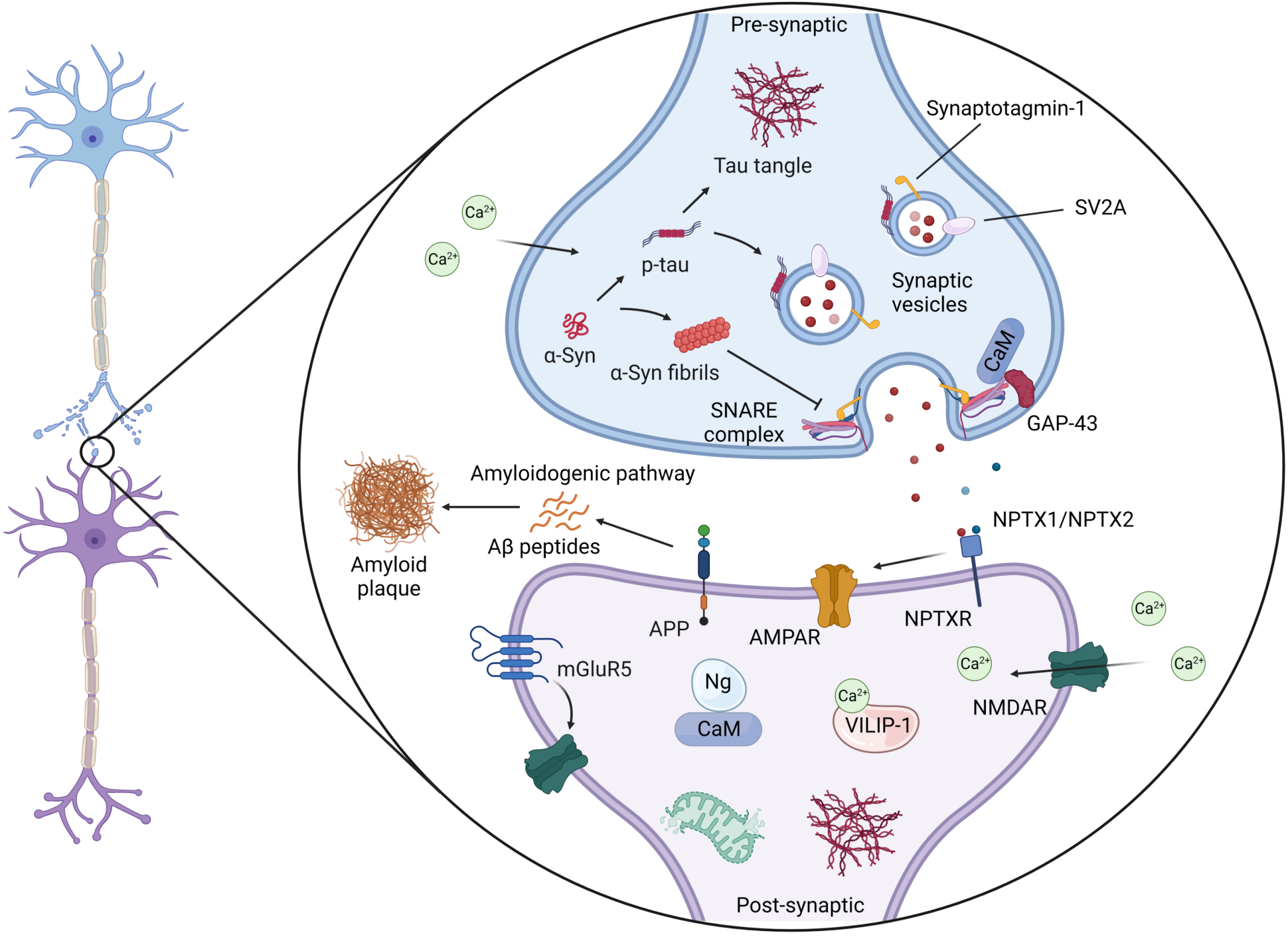
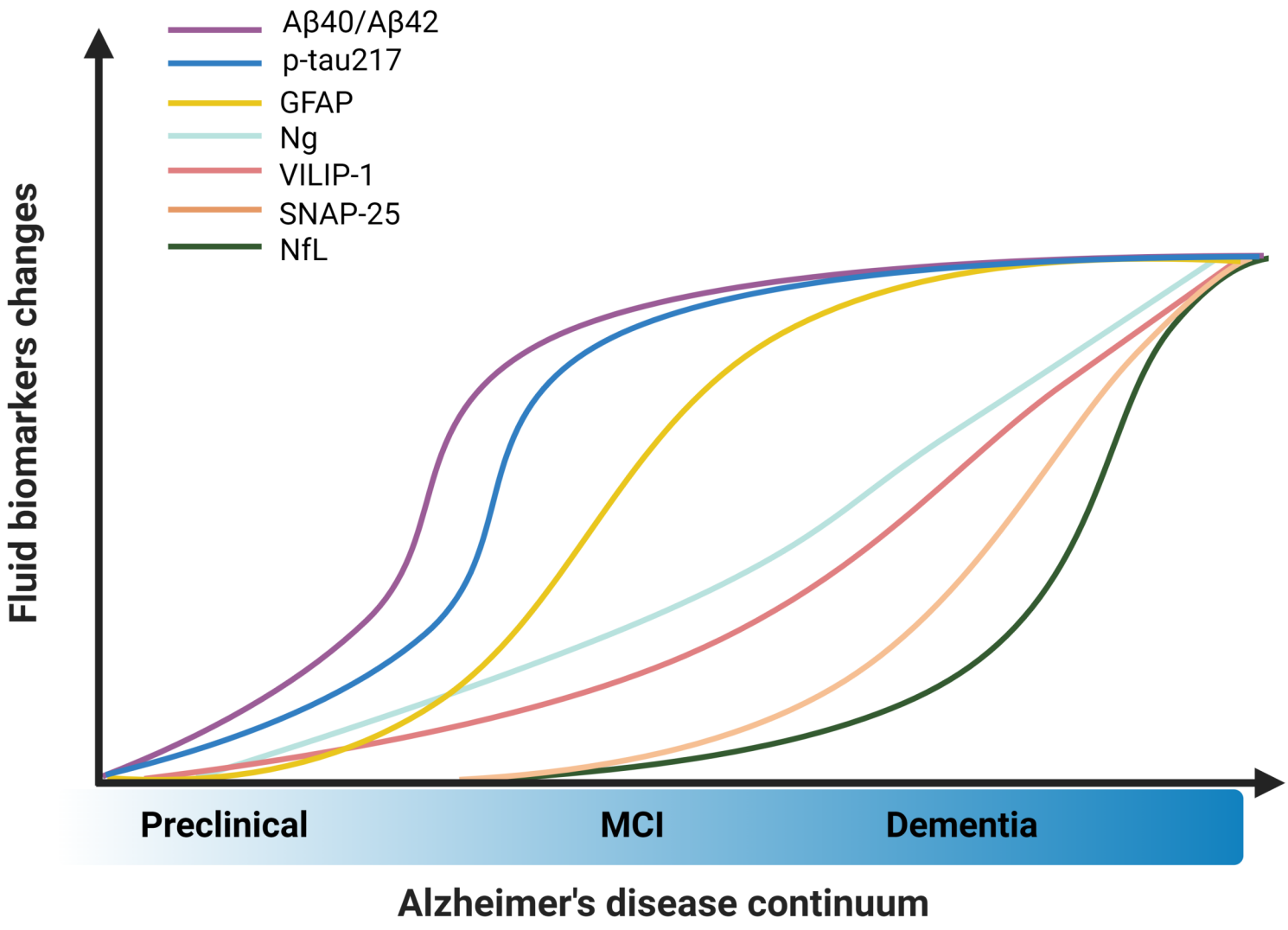

Pre-synaptic
Tau tangle
Synaptotagmin-1
SV2A
Ca2+
Ca2+
p-tau
Synaptic vesicles
α-Syn
α-Syn fibrils
CaM
SNARE complex
GAP-43
Amyloidogenic pathway
Amyloid plaque
Aβ peptides
NPTX1/NPTX2
APP
AMPAR
NPTXR
Ca2+
mGluR5
Ng
CaM
Ca2+
VILIP-1
Ca2+
NMDAR
Ca2+
Post-synaptic

FIGURE 2


Aβ40/Aβ42
p-tau217
GFAP
Ng
VILIP-1
SNAP-25
NfL
Fluid biomarkers changes
Preclinical
MCI
Dementia
Alzheimer's disease continuum


Pre-synaptic
Tau tangle
Synaptotagmin-1
SV2A
Ca2+
Ca2+
p-tau
Synaptic vesicles
α-Syn
α-Syn fibrils
CaM
SNARE complex
GAP-43
Amyloidogenic pathway
Aβ peptides
Amyloid plaque
NPTX1/NPTX2
APP
AMPAR
NPTXR
Ca2+
mGluR5
Ng
CaM
Ca2+
VILIP-1
Ca2+
Ca2+
NMDAR
Post-synaptic


Aβ40/Aβ42
p-tau217
GFAP
Ng
VILIP-1
SNAP-25
NfL
Fluid biomarkers changes
Preclinical
MCI
Dementia
Alzheimer's disease continuum