

BRAIN COMMUNICATIONS

REVIEW ARTICLE

Glial fibrillary acidic protein in Alzheimer's disease: a narrative review

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Astrocytes are fundamental in neural functioning and homeostasis in the central nervous system. These cells respond to injuries and pathological conditions through astrogliosis, a reactive process associated with neurodegenerative diseases such as Alzheimer's disease. This process is thought to begin in the early stages of these conditions. Glial fibrillary acidic protein (GFAP), a type III intermediate filament protein predominantly expressed in astrocytes, has emerged as a key biomarker for monitoring this response. During astrogliosis, GFAP is released into biofluids, making it a candidate for non-invasive diagnosis and tracking of neurodegenerative diseases. Growing evidence positions GFAP as a biomarker for Alzheimer's disease with specificity and disease-correlation characteristics comparable to established clinical markers, such as A β peptides and phosphorylated tau protein. To improve diagnostic accuracy, particularly in the presence of confounders and comorbidities, incorporating a panel of biomarkers may be advantageous. This review will explore the potential of GFAP within such a panel, examining its role in early diagnosis, disease progression monitoring and its integration into clinical practice for Alzheimer's disease management.

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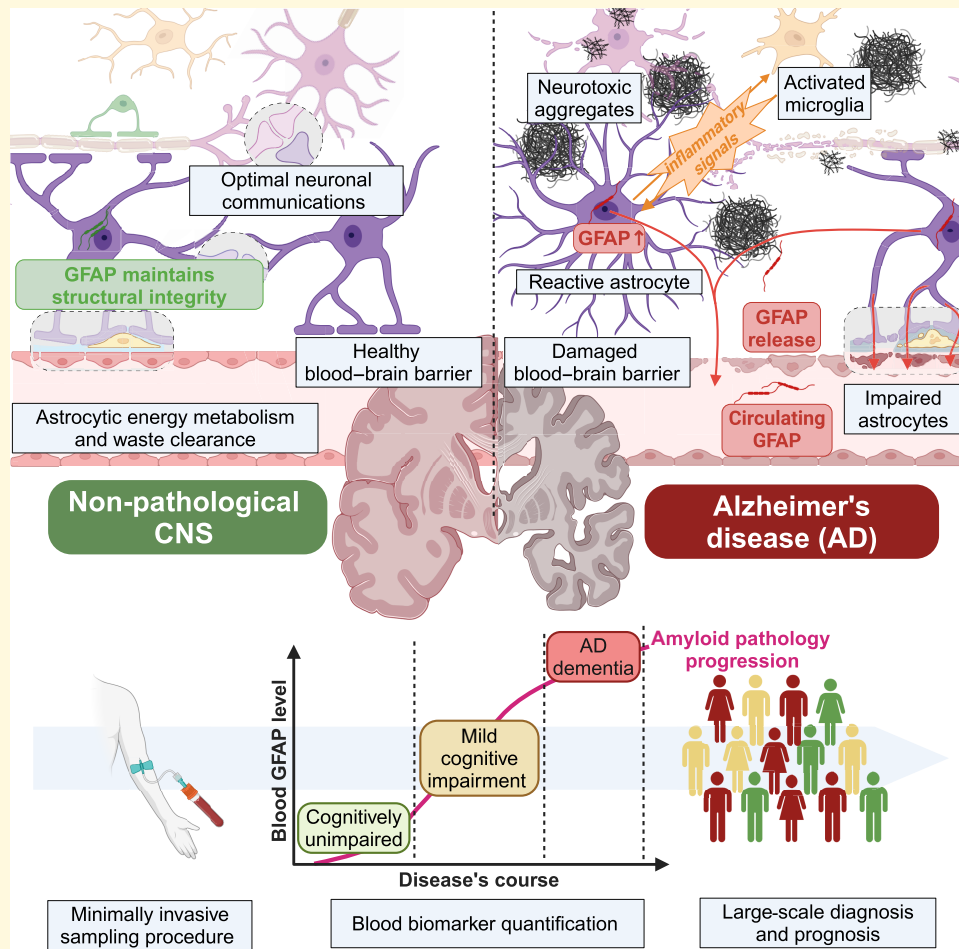
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Graphical Abstract



Introduction

With a projected prevalence of over 152 million cases by 2050, Alzheimer's disease is currently acknowledged as the prevalent form of dementia.¹ This neurodegenerative disease leads to severe memory loss and cognitive impairment. Key pathological features of Alzheimer's disease include intracellular neurofibrillary tangles of hyperphosphorylated tau (pTau) and extracellular senile plaques composed of amyloid- β peptides ($A\beta$) aggregates. These characteristic hallmarks are accompanied by glial response and chronic neuroinflammation.² Efforts to develop targeted therapies for Alzheimer's disease have consistently fallen short, leaving Alzheimer's disease among the ranks of incurable conditions. Clinical trials targeting $A\beta$ have shown low efficacy and significant side effects, resulting in an unfavourable risk-benefit ratio.³ The persistent challenge of finding treatments to prevent or slow down neurodegeneration without severe side effects highlights the multifaceted nature of the disease's pathogenesis. Consequently, research has shifted towards exploring and targeting alternative disease pathways.⁴ Despite the high accuracy of amyloid and tau-PET (positron

emission tomography) imaging as well as cerebrospinal fluid (CSF) quantification of established biomarkers, such as pTau and $A\beta$, their limited availability, high cost and invasiveness underscore the need for alternative biomarkers. Plasma biomarkers for Alzheimer's disease offer a timely and cost-effective alternative with less invasive sampling procedures.⁵ Recent developments in ultra-sensitive detection techniques have enabled the quantification of brain-derived proteins in blood, which reflect neurodegenerative processes. Among the extensively studied plasma biomarkers for Alzheimer's disease, notable candidates include $A\beta_{1-42}$, the $A\beta_{1-42}/A\beta_{1-40}$ ratio,⁶ tau proteins with phosphorylation at amino-acid positions 181, 217 and 231 (pTau-181, pTau-217 and pTau-231). These biomarkers reflect the pathological features of senile plaques and neurofibrillary tangles.⁷ Recently, novel blood biomarkers have emerged, including structural proteins from brain cells, such as neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP). NfL, a major constituent of axons, demonstrates a non-specific increase in response to brain injury or damage and correlates with neuroinflammation.⁸ GFAP, associated with astrogliosis, is linked to pathological processes in

Alzheimer's disease, particularly amyloid build-up, and neuroinflammation.⁹ The complexity and heterogeneity of neurodegenerative diseases complicate outcome prediction and patient care. The National Institute of Aging and Alzheimer's Association (NIA-AA) framework characterizes Alzheimer's disease as a biological construct, emphasizing diagnosis through its underlying pathological hallmarks and biomarkers. In light of recent advances, a proposed update to the 2018 [AT(N)] diagnosis criteria research framework document was presented at the Alzheimer's Association International Conference (AAIC) 2023 held in Amsterdam.¹⁰ Among the major updates, the ATN framework now incorporates biomarkers linked to reactive astrogliosis, acknowledging its role in Alzheimer's disease.¹¹ In response to neurodegenerative conditions, astrocytes become reactive, undergoing morphological and functional changes.¹² This includes GFAP overexpression and its release into the bloodstream.¹³ Reactive astrocytes produce inflammatory agents and are found surrounding amyloid plaques, suggesting their involvement in amyloid pathology and neuroinflammation.^{14,15} Recent studies suggest that astrogliosis occurs in the early stages of Alzheimer's disease, potentially preceding established hallmarks of the disease, such as A β aggregation and abnormal tau protein accumulation.¹⁶⁻¹⁸ GFAP's association with astrogliosis and emerging evidence of its involvement in the early stages of Alzheimer's disease highlight its potential as a biomarker. Plasma GFAP has shown superior diagnostic accuracy compared with GFAP measured in CSF, effectively distinguishing A β -positive from A β -negative individuals, predicting disease in at-risk populations and differentiating Alzheimer's disease from other neurodegenerative disorders such as frontotemporal dementia.¹⁹⁻²² Therefore, blood and CSF GFAP were added to the existing [AT(N)] framework as biomarkers for reactive astroglial response. Plasma biomarkers have demonstrated strong diagnostic and are now integrated with CSF and PET imaging biomarkers, serving as a powerful tool for large-scale screening and early diagnosis.²³ In clinical practice, blood biomarkers provide significant advantages for diagnosing, prognosing and monitoring Alzheimer's disease. They come with several advantages, including low invasiveness, simplicity in sample collection, affordability, speed of implementation and broad acceptance. This study explores the potential of GFAP as a blood-based biomarker for early Alzheimer's disease diagnosis and monitoring of disease progression. By offering insights into GFAP's role in reactive astrogliosis, we demonstrate its clinical relevance in enhancing diagnostic accuracy and advancing our understanding of neurodegenerative diseases.

GFAP as a biomarker of reactive astrogliosis

Astrocytes functions

Astrocytes are the most prevalent glial cells in the central nervous system (CNS) and are involved in a broad diversity of physiological mechanisms, essential to ensure brain health. The cytoskeleton of astrocytes is composed of numerous

intertwined fibrils called intermediate filaments, with GFAP being one of their major constituents.²⁴ The endfeet of astrocytes processes surround blood vessels, providing structural integrity to the blood-brain barrier (BBB).²⁵ Engaged in neurovascular coupling, they release signalling molecules to modulate vascular tone and provide proper oxygen and nutrient supply to activated neurons.²⁶ Astrocytes also actively participate in energy metabolism, ensuring glucose uptake from blood vessels. They constitute the major site of glycogen storage in the CNS and contribute to glycolysis and lactate secretion, which are essential for neuronal energy supply.²⁷ At the synaptic level, they contribute to neuronal communication and plasticity through specific ion channels, neurotransmitter transporters and receptors expressed on their surface.²⁸ They modulate neurotransmitter release, maintain ion homeostasis and clear metabolites from the brain to prevent toxic accumulation.²⁹⁻³¹ Aquaporin 4 (AQP4) is a water channel, localized in astrocytic membranes, facilitating water exchange between brain interstitial fluid and CSF, indirectly supporting the clearance of protein and metabolites from the brain.³²⁻³⁴ Beyond their role in metabolic processes, astrocytes also play a key role in developmental stages. They establish molecular boundaries, initiate synaptic maturation, axonal development and angiogenesis.^{35,36}

Astrocytes divide into subtype populations that form clusters, each with different localization, function and molecular composition within the CNS. Protoplasmic astrocytes, predominantly found in the grey matter, have numerous and short processes that provide extensive contact with neurons, regulating extracellular neuronal microenvironment and contributing to synapse function and plasticity.³⁷ Conversely, fibrous astrocytes, located in the white matter, have fewer but longer processes and are mainly involved in axonal myelination and metabolic support.³⁸ However, recent findings shown that astrocytic heterogeneity is more complex than previously acknowledged reflecting their fine adaptation to local environments.³⁹ Beyond morphological differences and spatial distributions, astrocyte subtypes exhibit transcriptomic and functional diversity across brain regions.⁴⁰ Hasel *et al.*⁴¹ recently performed RNA sequencing and spatial transcriptomics to identify and characterize a marginal inflammatory astrocyte subtype that specifically expresses myocilin and genes involved in immune reactivity.⁴² These astrocytes are located at the brain surface beneath the pia mater and constitute the *glia limitans superficialis*.⁴¹ Additionally, other *glia limitans* astrocytes surround endothelial cells of the brain vasculature with their endfeet and constitute the *glial limitans vascularis*, providing mechanical support and maintaining the integrity of the BBB.⁴³ These observations support the hypothesis that *glia limitans* astrocytes act as sentinels, poised to respond to inflammatory signals and may participate in the spread of the disease if disrupted.⁴⁴ Further studies have identified other region-specific cluster populations of astrocytes across cortical layers.⁴⁵ Their exact roles in health and disease remain unclear and require further investigation to understand

regional susceptibility to diseases such as Alzheimer's disease or Parkinson's disease, in which astrocytes are extensively implicated.

Overall, astrocytes are multifaced indispensable components of the CNS, orchestrating a myriad of functions essential for neuronal health and synaptic connectivity while adapting to the needs of their local environment. Their intricate interactions with neurons and the vasculature underscore their pivotal role in maintaining brain homeostasis and supporting proper brain function throughout life.

GFAP structure

GFAP gene transcription and isoforms

The human *GFAP* gene, located on chromosome 17q21, was identified through mouse, rat⁴⁶ and human cDNA cloning studies.⁴⁷ This gene comprises nine exons and eight introns,

which are further diversified by the incorporation of four alternative exons and two alternative introns.⁴⁸ Different protein isoforms are produced from the *GFAP* gene due to alternative splicing processes following RNA transcription.⁴⁹ The complete sequence of *GFAP* was elucidated in the early 1990s using cDNA cloning experiments conducted on mouse brains, revealing a predicted full length of 432 amino acids with a molecular weight of 49.9 kDa.^{50,51} To date, 12 human proteoforms have been identified (Fig. 1).⁴⁹ Among these, GFAP α is the predominant isoform, accounting for approximately 90% of total GFAP expression within the brain, spinal cord and peripheral nervous system (PNS).⁵² Further studies, utilizing RT-PCR assays, revealed that GFAP β isoform was not only expressed in brain cells but also within Schwann cells of the PNS and in non-neural cells.^{46,53,54} Initially discovered in mouse brain and spleen,

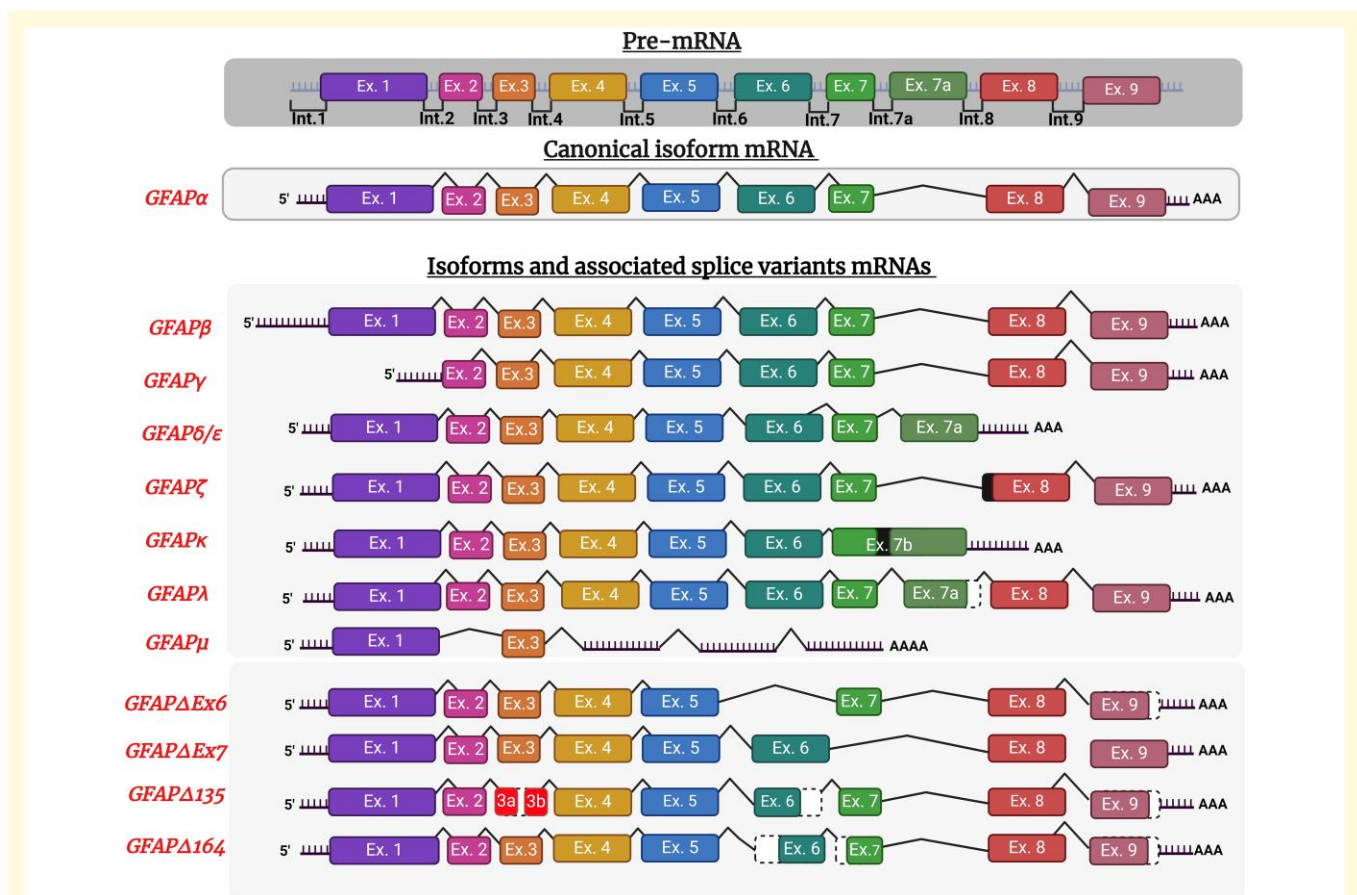


Figure 1 *GFAP* alternative splicing. Pre-mRNA and isoform mRNAs of *GFAP* are represented in the figure. *GFAP* is the human gene encoding for human glial fibrillary acidic protein (GFAP) and contains nine exons presented in coloured boxes and separated by untranslated introns. Exons 1, 4 and 5 are constitutive for most isoforms (except GFAP μ), while the others undergo alternative splicing leading to 12 human identified isoforms. GFAP α is the canonical transcript and most abundant isoforms of GFAP. Exon 1 contains start codon ATG and encodes N-terminal domain of GFAP, except for GFAP β where the transcription site is about 169 nucleotides upstream. Several isoforms lack exons. GFAP γ lacks exon 1 in the N-terminal domain, and GFAP μ transcripts contain only exons 1 and 3 lack most of the core coil domains. GFAP δ/ϵ and GFAP κ lack exons 8 and 9 encoding for the last coil and C-terminal domains. Conversely, splicing variant of several isoforms include additional exons like GFAP λ and GFAP δ/ϵ containing exon 7a after exon 7 and GFAP κ containing exon 7b containing both exon 7, intron 7a and exon 7a. GFAP +1 isoforms category gathers GFAP Δ Ex6, Δ 7, Δ 135 and Δ 164 isoforms with exhibits several alternatives changes regarding canonical form. Among those, complete or partial deletion of exon 6, exon 7 or exon 3 and exon 9 shortening. Deletions are represented by black-dotted white boxes and intron transcription by black-filled boxes.

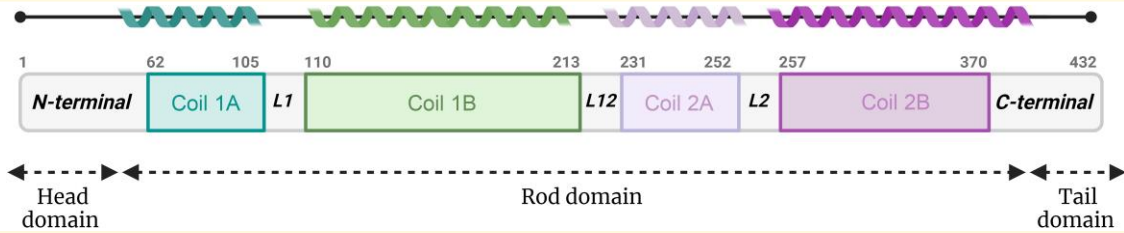


Figure 2 Canonical GFAP 3D structure. Canonical GFAP is a 432 amino-acid long protein composed of two core coil domains respectively divided into two subparts (A and B). Together, the four coil subparts structured in alpha helix constitutes the rod-domain situated between head and tail domains. The different coil parts are connected with three linkers (L1, L12 and L2).

GFAP γ mRNA was subsequently also observed in human brain, spleen and bone marrow.⁵⁵ GFAP β transcription start site is 169 nucleotides upstream GFAP α putative start site. GFAP γ transcript lacks exon 1 and its transcription start site is thought to be 130 nucleotides before exon 2 starts, in intron 1.⁵⁵ Furthermore, GFAP δ transcript was identified in rat cells,⁵⁶ and its human homolog, GFAP ϵ , was independently identified in a presenilin-binding partner study.⁵⁷ GFAP δ/ϵ is preferentially expressed by neurogenic astrocytes of the subventricular region.^{58,59} GFAP δ mRNA includes the transcription of an additional exon (exon 7a) and exhibits a completely different C-tail, lacking exons 8 and 9.⁵⁶ Additionally, GFAP ζ with a longer transcript comprises the transcription of the last 284 nucleotides of intron 8 before exon 9 and was found to be extensively expressed in mouse brain.⁶⁰ GFAP κ transcripts reported in mouse and pig cortex, cerebellum and striatum present an additional exon (exon 7b) before 8 exon which is composed of exon 7, intron 7a and exon 7a and exhibit a distinctive shortened and modified C-terminal tail.⁶¹ Although mRNAs were identified, there is currently no evidence for human protein expression of GFAP β , GFAP γ , GFAP κ and GFAP ζ yet.^{60,62} More recently, GFAP λ and GFAP μ transcripts were discovered within human brain and spinal cord. GFAP λ like GFAP δ contains exon 7a but keeps exons 8 and 9 transcriptions, while GFAP μ only contains exons 1 and 3 with a premature termination codon following exon 3.^{63,64} Moreover, GFAP has four alternative isoforms collectively termed GFAP + 1 present in astrocyte subgroups associated with single nucleotide frame-shift variants: GFAP Δ Ex6, GFAP Δ 164, GFAP Δ 135 and GFAP Δ Ex7.⁶⁵ Increased levels of specific GFAP isoforms have been reported over the course of amyloid pathology. The work of Kamphuis *et al.*⁶⁶ on Alzheimer's disease brain donors revealed high expression of GFAP δ in reactive astrocytes surrounding plaques and spreading within the hippocampus where the disease severity is increased. In the same study, GFAP transcript levels for GFAP α , GFAP δ , GFAP ζ , GFAP κ , GFAP Δ 135 and GFAP Δ Ex7 were also found to be increased and correlated with brain amyloid build-up. Moreover, GFAP + 1 isoforms expression was observed in specific subtypes of non-reactive astrocytes, characterized by large cell bodies and long processes, with elevated expression in Alzheimer's disease. This astrocyte population also responded to A β -related cellular

stress *in vitro*. However, the differential effects of GFAP isoforms in astrogliosis remain largely underexplored, necessitating further investigations to clarify their implications.

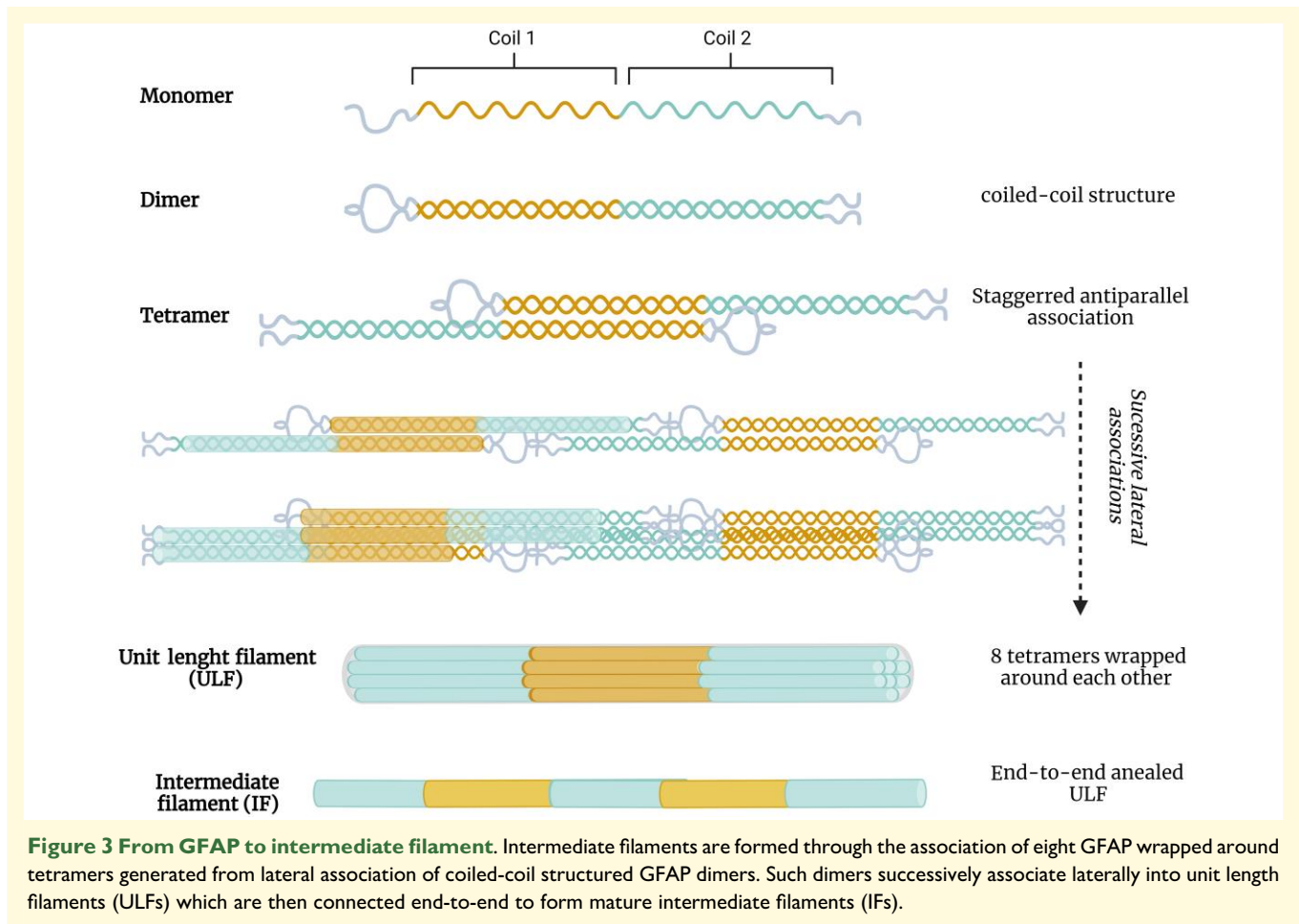
GFAP protein structure and post-translational modifications (PTMs)

The 3D structure of GFAP was elucidated through X-ray crystallography studies.⁶⁷ It consists of four alpha-helical segments (1A, 1B, 2A and 2B) forming the 'rod-domain', surrounded by flexible non-helical N-terminal head and C-terminal tail domains (Fig. 2).⁶⁸ Moreover, GFAP can undergo several post-translational modifications influencing its structure and properties.⁶⁹ GFAP exhibits 16 phosphorylation sites with phosphorylation at serine 8 and 13 being involved in GFAP polymerization as well as the stability of intermediate filaments.^{70,71} Additionally, GFAP contains 28 arginine residues, which can undergo citrullination. Notably, this modification has been associated with several auto-immune or inflammatory conditions such as rheumatoid arthritis or multiple sclerosis, but not with Alzheimer's disease.^{72,73} Furthermore, the single cysteine in the GFAP sequence might be subject to lipoxidation, potentially impacting the assembly of GFAP in the intermediate filament network.⁷⁴

GFAP functions

GFAP ensures astrocytes' structural integrity

GFAP was first identified on plaques with fibrous astrocytes and demyelinated axons obtained from the brains of individuals with multiple sclerosis.²⁴ Along with vimentin, nestin and synemin, GFAP is one of the principal building blocks of intermediate filaments. Their specific localization in intermediate filaments was determined with immunostaining in cultured cells and tissues.⁷⁵ The first step of intermediate filament assembly is the formation of parallel dimers in a coiled-coil structure, followed by the lateral antiparallel connection of dimers into tetramers and octamers. Subsequently, octamers associate laterally and are wrapped around each other to generate one unit length filament (ULF). Finally, ULFs associate into nanometer-long filaments to form mature filaments.^{68,76} Notably, GFAP can also form homodimers or heterodimers with vimentin.⁷⁷ The entire formation process is presented in Fig. 3. As a type III intermediate filament protein, GFAP plays a crucial part in providing mechanical



support, reinforcing astrocytes cytoskeleton and scaffolding for enzymes and organelles, as well as sensing mechanical cues from the extracellular environment.⁷⁸

Over the past decades, several mouse models have been developed to explore GFAP's extended physiological role. Gomi *et al.*⁷⁹ reported that GFAP-null mice developed normally, showing no visible sign of deformity, infertility or developmental abnormalities compared with wild-type mice. This observation, corroborated by western blots in the same study, suggested that GFAP may not be essential for CNS development under physiological conditions. Although GFAP-null mice develop without visible abnormalities, GFAP is critical for maintaining CNS integrity, especially under conditions of aging or stress. For instance, other findings showed that aging GFAP-null mice (from 6 to 18 months) exhibited poorly vascularized and altered white matter due to impaired astrocytic scaffolding.⁸⁰ Another study reported long-term deficits in CNS axon myelination and increased BBB permeability.⁸⁰ More substantial deficiencies become evident following injury, especially head traumatism. One research group studied the consequences of simulated head concussion on sedated mice by subjecting them to weight drop impacts without head support.⁸¹ Among the 15 GFAP-null mice, 12 died primarily due to upper cervical spinal cord injury leading to respiratory arrest

while all 14 wild-type mice survived. This suggests that GFAP within the intermediate filaments networks plays a role in maintaining the elasticity and structural strength of CNS. GFAP-deficient animals also exhibited increased sensitivity to cerebral ischaemia, with significant neuronal damage observed. Overall, these findings indicate that astrocytes provide brain protective roles, which are compromised under GFAP deficiency. GFAP interacts with various proteins and regulatory compounds, involved in intermediate filaments architecture and dynamics, including presenilin, LAMP-2A or α B-crystallin.^{57,82,83} Immunostaining experiments demonstrated that vimentin persists in the mature brain in the absence of GFAP, suggesting that vimentin compensates for the loss of GFAP in regions where it is typically expressed.⁸⁴ Pekny *et al.*⁸⁵ observed that GFAP(-/-) and vimentin(-/-) mice still developed intermediate filaments, whereas GFAP(-/-) and vimentin(-/-) mice completely lacked intermediate filaments in their astrocytes cytoskeleton both *in vivo* and *in vitro*. These findings highlight vimentin's ability to form intermediate filaments with either GFAP or nestin as a mandatory partner. However, nestin alone cannot assemble intermediate filaments, as corroborated by a subsequent study.⁸⁶

GFAP expression is notably higher in fibrous astrocytes compared with protoplasmic astrocytes.⁸⁷ Hasel *et al.*⁴¹

also reported that along with reactive gene expression, Myoc+ astrocytes in the *glia limitans* exhibit elevated GFAP expression, surpassing baseline levels seen in other astrocyte subtypes. Furthermore, not all astrocytes express detectable levels of GFAP in healthy tissues. The elevated levels of GFAP in *glia limitans* astrocytes suggest that these cells may be 'pre-reactive', positioning them among the first responders to CNS insults and injuries.³¹

GFAP and reactive astrocytes

Astroglial gradual response to CNS injury

Astrocytes, beyond their diverse roles under normal conditions, respond to stress and injury signals of danger through astrogliosis.¹² Initially, reactive astrogliosis is a protective process involving multiple cellular and molecular responses to CNS injury. Key participants include neuronal cells such as astrocytes, oligodendrocytes and neurons, as well as non-neuronal cells like microglia, pericytes and perivascular fibroblasts. Additionally, extrinsic cells infiltrating from the bloodstream such as leukocytes, platelets and fibrocytes can also contribute to this response.⁸⁸ Collectively, these cells have the ability to affect and modulate each other's activity through intricate molecular signalling pathways involving various neurotrophic factors and cytokines.⁸⁸ Activated astrocytes regulate inflammatory response by controlling the entry, activation and trafficking control of peripheral immune cells such as leukocytes. Through direct cell-to-cell communication, astrocytes perform functions akin to those of antigen-presenting cells. Alternatively, they engage in indirect signalling by secreting soluble molecules, thereby actively contributing to the cerebral innate immune system.^{89,90} Astrogliosis is a heterogeneous phenomenon with its intensity and characteristics directly related to the severity of the damage. It ranges from mild astrogliosis, with reversible changes in gene expression and cellular hypertrophy, to severe astrogliosis, marked by enlarged glial scars and permanent tissue remodelling.⁹¹ An essential hallmark of astrogliosis is the overexpression of GFAP. Elevated GFAP levels correlate directly with the severity of neural damage, leading to structural hypertrophy of reactive astrocytes. In cases of severe and widespread brain injuries, astrocytes undergo extensive proliferation and form physical and chemical barriers surrounding lesion sites.^{92,93} This response is critical for containing damage and preventing its propagation to healthy tissues. The upregulation of GFAP acts as a marker of astrocyte activation following neural injury, underscoring their enhanced protective functions.

GFAP overexpression in astrogliosis

GFAP expression evolves during CNS maturation and aging. As previously described, vimentin is the major constituent of juvenile neuron tissue and is gradually replaced by GFAP during development.^{94,95} The onset of GFAP expression varies depending on the tissue under consideration. However, the number of cells expressing GFAP tends to rise with gestational age, particularly in the latter half of the gestational period.⁹⁶⁻⁹⁸ GFAP is first expressed in neural stem cells

within the ventricular zone, later extending to the subventricular zone, where it persists postnatally and throughout adulthood.^{99,100} During aging, GFAP transcription and expression increase, primarily due to oxidative stress, a hallmark of normal aging.^{101,102} Nichols *et al.*¹⁰³ reported increased transcriptional activity during aging, particularly in the hippocampus and frontal and temporal cortex regions. These areas are often impacted by neurodegenerative diseases associated with reactive astrogliosis. One of the primary functions of GFAP within the CNS is to provide structural support to astrocytes. Under pathological conditions such as inflammation, neurodegeneration or traumatic brain injury, GFAP expression is elevated leading to astrocyte changes in morphology through intermediate filament rearrangement. The effects of GFAP overexpression were investigated in transgenic mice carrying human genomic clones of the *GFAP* gene resulting in GFAP overexpression.¹⁰⁴ The engineered mice presented hypertrophic astrocytes with intracellular intermediate filaments aggregates and inclusion bodies identical to Rosenthal fibres found in Alexander's disease.^{105,106} GFAP overexpression, while essential for promoting tissue repair and containing damage in the CNS, can also lead to the formation of glial scars and disruption of neural circuits, ultimately contributing to cognitive decline. This dual role underscores the delicate balance between the protective and potentially harmful effects of astrocyte activation.¹⁰⁷

Astrogliosis in Alzheimer's disease

Astrocyte dysfunction is increasingly recognized as a key factor in the pathophysiology of Alzheimer's disease and other neurodegenerative disorders, including Parkinson's disease, multiple sclerosis, Alexander's disease and Huntington's disease.¹⁰⁸ Emerging evidence suggests that reactive astrogliosis may precede classical pathological hallmarks such as A β deposition and abnormal tau protein aggregation.¹⁰⁹ The astroglial response is consistently observed and progressively intensifies as Alzheimer's disease progresses.¹¹⁰ This response leads to significant changes in astrocyte morphology and activity, profoundly impacting both disease severity and the healing process.¹¹¹ Throughout neurodegenerative disease progression, reactive astrocytes have been reported to transition from a supportive role to acquiring a toxic role, with the degree of gliosis closely correlating with neurodegeneration severity. A GFAP immunoassay study on brain tissues revealed that the most pronounced glial response occurs in the hippocampus within the temporal lobe, the region where Alzheimer's disease first induces neurological damage.¹¹²

Under pathological conditions, activated astrocytes upregulate the production of A β and release pro-inflammatory mediators, thereby initiating neuroinflammation and neurodegeneration.¹¹³ Accumulating A β , similar to alarmins, can attract astrocytes to the vicinity of the lesion through chemotactic molecules, affecting astrocytes morphology and functions and leading to progressive brain damage. Wyss-Coray *et al.*¹¹⁴ cultured mouse astrocytes and observed their progressive build-up around plaques in response

to their release of monocyte chemoattractant protein-1. Their findings also indicated that astrocytes might degrade A β by binding to and phagocytosing A β deposits. Neuropathological findings further support the strong association between plaques and astrogliosis, with reactive astrocytes following the spatial distribution of A β plaques.¹¹⁵ Kamphuis *et al.*⁶⁰ in line with previous observations by Xu *et al.*¹¹⁶ reported that GFAP-null astrocytes, unlike normal astrocytes, exhibited a delayed and reduced capacity to form tight and extensive boundaries around amyloid aggregates. These findings underscore the critical role of GFAP in facilitating effective confinement of neurodegenerative lesions through astrogliosis. In contrast to amyloid plaques, only few studies have examined the relationship between reactive astrocytes and tau tangles.¹¹⁷ It is thought that reactive astrocytes interact with tau protein to a lesser extent, predominantly in the later stages of Alzheimer's disease. Astrocytes appear to abnormally internalize tau protein, leading to the formation of intracellular neurofibrillary aggregates that contribute to the propagation of pathological tau across the brain.¹¹⁸ Tau aggregates further induce astrocyte senescence with high mobility group box 1, a death-associated inflammation marker. It is also important to consider that throughout the progression of Alzheimer's disease, A β and tau pathology experience a complex and intricate association.¹¹⁹ Nonetheless, the exact mechanisms underlying this relationship remain incompletely understood, and it is unclear whether this association exists independently of the amyloid plaques.¹²⁰ Astrogliosis contributes to several mechanisms directly linked to neurodegeneration, exacerbating its pathological features. Pronounced reactive astrogliosis leads to the extensive production of reactive oxygen species (ROS), particularly hydrogen peroxide (H₂O₂). This increase in ROS contributes to the accumulation of pathological tau, cerebral atrophy and cognitive decline through oxidative stress.¹²¹ Neuronal hyperexcitability, characterized by an abnormal increase in neuronal activity, is another pathological feature in neurodegeneration. This hyperexcitability, potentially resulting from impaired ion channel function, synaptic irregularities or neurotransmitter imbalances is closely associated with neuronal death and cognitive decline.¹²²

Neuroinflammation initially serves as a protective response, defending the brain against potential threats and counteracting disease progression. However, as Alzheimer's disease advances, chronic inflammation persists and exacerbates neurodegenerative damage.¹²³ This process involves a complex multicellular network, including microglia, astrocytes, oligodendrocytes and neurons, which interact through a cascade of signalling pathways that ultimately amplify inflammation and contribute to neuronal damage.⁸⁸ Following A β deposition, activated microglia and reactive astrocytes engage in tightly regulated communication, significantly contributing to the neuroinflammatory response. This interaction is a hallmark of Alzheimer's disease's progression and highlights the pivotal role of these glial cells in sustaining inflammation. A major factor sustaining this

neuroinflammation is the dysregulation of calcium signalling, which establishes a vicious cycle that perpetuates glial activation and drives neurodegeneration.¹²⁴ Elevated levels of calcineurin in astrocytes and microglia induce the production of pro-inflammatory factors such as cytokines including interleukins, tumour necrosis factor- α and ROS. These factors further disrupt Ca²⁺ pathways, leading to progressive neuronal loss and subsequent cognitive impairment.¹²⁵ In 5xFAC, an A β -bearing mouse model, calcineurin hyperactivation impaired astrocytic glutamate transporter function, causing deficient glutamate buffering, which is known to have excitotoxic effects.¹²⁶ Additionally, oligodendrocytes exposed to these inflammatory signals exhibit impaired survival and function, disrupting myelination and exacerbating white matter damage, further contributing to cognitive decline.¹²⁷

These findings highlight a significant association between reactive astrogliosis and Alzheimer's disease in which all brain cells are involved and interact in a deleterious synergistic manner that is still not fully understood. Consequently, astrocytes emerge as a highly promising target for the development of innovative therapeutic candidates and form a potential source of biomarkers for diagnosis and prognosis. However, astrogliosis represents only one aspect of the complex and dynamic neurodegenerative process. The multifaceted role of astrocytes in neurodegeneration is illustrated in Fig. 4.

Relevance of GFAP as a clinical biomarker for Alzheimer's disease

GFAP leakage in the bloodstream

GFAP is primarily localized intracellularly, but various mechanisms can induce its release into the extracellular space and subsequent entry to the bloodstream. The mechanisms underlying GFAP leakage into the blood under neurodegenerative conditions are complex and are not yet fully understood. Astrocytic damage or death due to astrogliosis and neuroinflammation is a prominent mechanism that may release GFAP into biofluids.^{127,128} Astrocyte endfeet, which align along brain capillaries and the BBB, may facilitate GFAP release into the bloodstream, particularly when the BBB is compromised.¹²⁹ Disruption of the BBB, which occurs naturally with aging and is observed in early Alzheimer's disease before the onset of dementia or neurodegeneration, could lead to increased levels of circulating GFAP and other soluble brain proteins.¹³⁰ Multiple factors contribute to BBB disruption in Alzheimer's disease, including neuroinflammation, the presence of APOE4 allele and amyloid deposition.⁴³ The elevated risk of Alzheimer's disease associated with APOE4 is partly due to its role in enhancing the permeability of brain vessels. Conversely, APOE2 is considered protective, maintaining BBB integrity.¹³¹ Dysregulation of calcium signalling and the release of inflammatory markers associated with neuroinflammation can also compromise BBB function by inducing the loss of tight junctions.¹³² In addition to its effects on the BBB, the APOE4

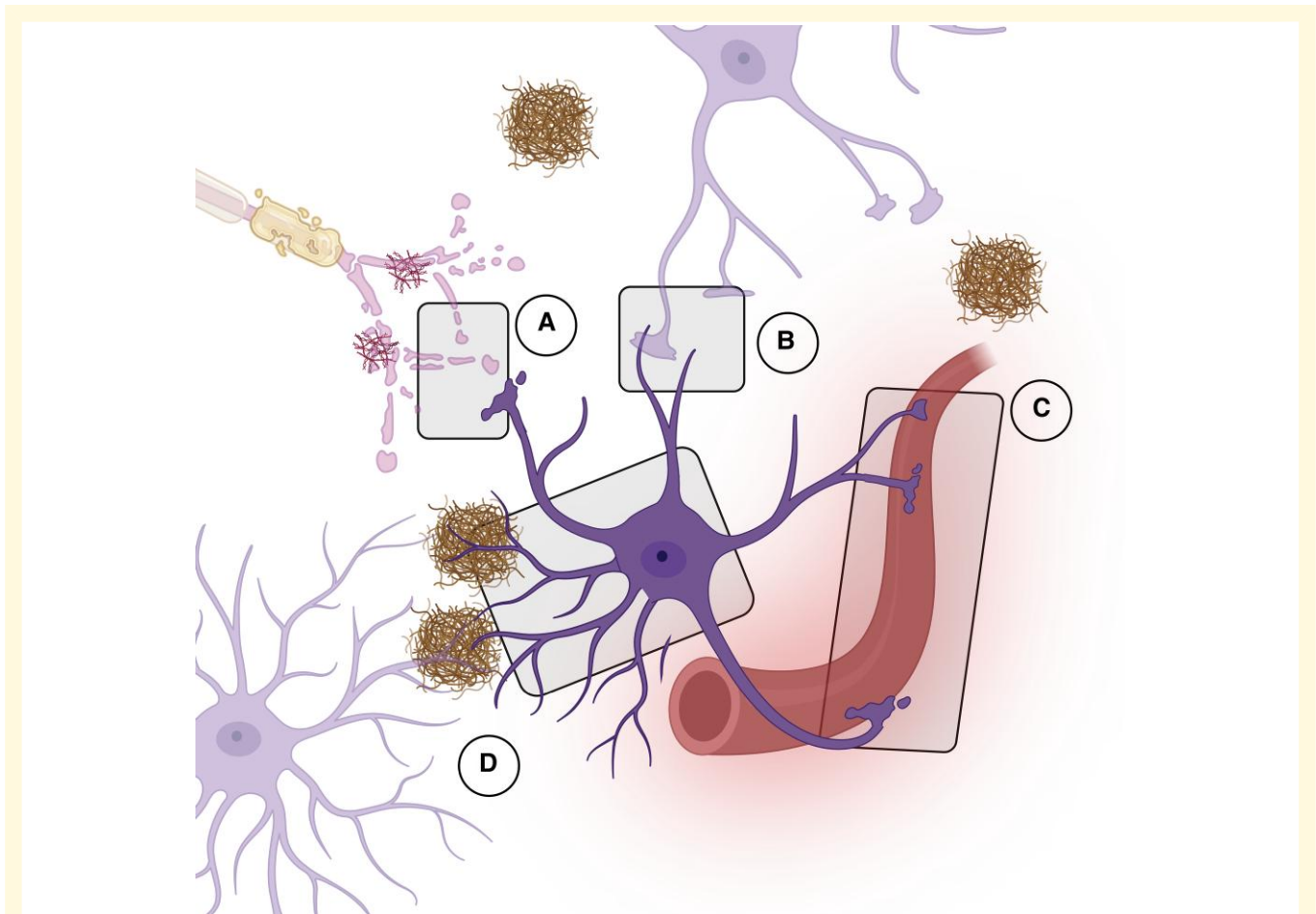


Figure 4 Astrocytes roles and modifications in Alzheimer's disease. Due to their multifaced functions and implications in various brain structures, reactive astrocyte modifications deeply impact brain physiology at several levels. **(A) Tripartite synapses:** Alteration of neurotransmitter and glutamate pathways, modification of water and ion channel transport and release of inflammatory substances leading to synaptic dysfunctions, neurotoxicity and neuroinflammation; **(B) Astrocytes tight junction:** Irregular ion transient (Ca^{2+} , K^{+}) and decreased gap junctions coupling; **(C) Astrocytes endfeet surrounding the blood–brain barrier (BBB):** Release of overexpressed GFAP due to astrocytes impairment associated with BBB disruption and immune-cell massive infiltration; **(D) Astrocytes morphology:** Astrocytes structural atrophy and proliferation confining amyloid lesions and inducing $\text{A}\beta$ clearance through the glymphatic pathway. Neurofibrillary tangles internalization.

allele is strongly associated with cerebral amyloid angiopathy, a condition characterized by amyloid deposition along brain vessel walls. This condition is commonly observed in Alzheimer's disease and contributes to the vascular pathology that exacerbates neurodegeneration. Increased secretion of $\text{A}\beta_{1-42}$ and $\text{A}\beta_{1-40}$ as well as reduced clearance due to glymphatic system impairment, induces aggregation within the parenchyma and along brain vasculature. This aggregation leads to BBB leakage, vessel occlusion or rupture, collectively worsening Alzheimer's disease symptoms and cerebral impairment.¹³³ The glymphatic system, a structured network involving astrocytes, may also provide a route for GFAP to enter the circulation.¹³⁴ This system is essential for clearing the brain of metabolic waste and potentially harmful substances. Dysfunction of the glymphatic system in neurodegenerative diseases hinders the removal of pathogenic proteins like $\text{A}\beta$, leading to further aggregation.¹³⁵ This disruption exacerbates neuroinflammation and oxidative stress, thereby driving disease progression.^{136,137}

Recent evidence suggests that reactive astrocytes may also release GFAP through exocytosis, secreting small vesicles into extracellular space. These vesicles, capable of crossing the epithelial barrier of blood vessels, could contribute to the presence of circulating GFAP.¹³⁸

GFAP as a clinical biomarker

Although pTau and $\text{A}\beta$ are the most commonly used blood biomarkers for assessing amyloid pathologies and tauopathies in Alzheimer's disease, recent studies have identified GFAP as a reliable alternative and potentially more sensitive indicator of pathological states. The implementation of next-generation immunoassays over the last decade has facilitated the rapid and reliable measurement of protein biomarkers in blood samples, enhancing the utility of CNS-derived markers. Given the pivotal role of astrogliosis in the pathogenesis of Alzheimer's disease, growing evidence from large patient cohorts suggests that GFAP is a reliable biomarker for distinguishing Alzheimer's disease from other neurodegenerative

disorders even at early stages.^{9,19} Furthermore, GFAP is highly brain-specific and is not extensively secreted into biofluids under physiological conditions, reinforcing its relevance as a neurodegenerative brain disease biomarker.¹³⁹

Blood GFAP levels differentiate Alzheimer's disease from other neurodegenerative dementias

In 2013, within a moderate-size study including three 331 patients with different neurological conditions including brain concussion, infection, cancer or various dementias, Mayer *et al.*¹⁴⁰ found that GFAP was reliably useful only for diagnosing intracranial haemorrhage and extensive intracranial bleeding. In other disease groups and controls, GFAP levels were close to the detection limit. Nevertheless, this study used a prototype Elecsys immunoassay (Roche) with a limit of quantification (0.05 µg/mL) considerably higher than the lower limit of quantification achieved with current technologies, such as single-molecule array (Simoa) platform (0.70 pg/mL, Quanterix), which might explain the lack of significant differences in GFAP levels for other pathologies. In 2018, the Brain Trauma Indicator, an assay measuring GFAP together with ubiquitin terminal-hydrolase-L1 in blood received Food and Drug Administration approval for assessing traumatic brain injury severity aiding the decision-making for CT scan use.¹⁴¹ With technical advances and the advent of fourth-generation immunoassays over the past decade, increasing evidence supports the diagnostic utility of plasma GFAP in distinguishing Alzheimer's disease from other dementias. Despite reactive astrogliosis being a common pathological hallmark in both frontotemporal dementia and Alzheimer's disease, significant differences were observed regarding blood GFAP level suggest that astrogliosis may differ between these pathologies. Oeckl *et al.*²¹ used Quanterix Simoa GFAP Discovery kit to measure serum GFAP and observed a 2-fold increase in Alzheimer's disease ($n = 230$) group compared with the behavioural frontotemporal dementia group ($n = 140$, $P < 0.001$) and controls ($n = 129$, $P < 0.001$). Serum GFAP was also increased between early-stage mild cognitive impairment (MCI) individuals ($n = 111$) compared with those with behavioural frontotemporal dementia ($P < 0.01$) and controls ($P < 0.001$). This correlation was consistent across four sub-cohorts (Coimbra, Munich, Ulm and Nijmegen). Serum GFAP differentiated Alzheimer's disease patients from control with an area under the curve (AUC) of 0.87 and from behavioural frontotemporal dementia with an AUC of 0.81, highlighting GFAP's potential as a differential biomarker. Further studies have demonstrated the utility of plasma GFAP in differentiating other neurodegenerative dementias such as supranuclear palsy. Baiardi *et al.*¹⁴² found that plasma GFAP, measured using the Simoa SR-X platform, could differentiate Alzheimer's disease from frontotemporal dementia and supranuclear palsy with respective receiver operating characteristic (ROC) curve AUCs of 0.82 and 0.77, respectively. However, differentiation between Alzheimer's disease and corticobasal syndrome (AUC 0.62) or dementia with Lewy bodies (AUC 0.58) was less effective. Overall in this study,

plasma GFAP showed strong performances, with AUCs ranging from 0.74 to 0.94, with the most consistent increases observed in Alzheimer's disease.

Blood GFAP surpasses CSF GFAP

Unlike other common Alzheimer's disease biomarkers, which typically perform better in CSF, plasma GFAP outperforms CSF GFAP in distinguishing between Aβ-positive and Aβ-negative individuals.^{143,144} Benedet *et al.*¹²⁹ analysed three major cohorts: TRIAD (Canada, $n = 300$), ALFA + (Spain, $n = 384$) and Paris Labroisière BioCogBank (France, $n = 187$) using Simoa HD-X Single-Plex Assay (Quanterix). Their results showed that plasma GFAP provided greater accuracy than CSF GFAP in differentiating Aβ-positive from Aβ-negative individuals, with ROC curve AUCs ranging from 0.69 to 0.86 for plasma and 0.59 to 0.76 for CSF.¹⁹ This outcome is unexpected, as blood biomarkers for neurological conditions have traditionally been substitutes for CSF biomarkers. However, as discussed, the superior performance of plasma GFAP may reflect its more direct association with astrocytic activity and neurodegeneration, which might not be as readily captured through CSF measurements.

Blood GFAP correlates with amyloid build-up but not with tau pathology

Multiple studies suggest that plasma GFAP may be a biomarker for Aβ pathology (A+) rather than tau pathology (T+). In the ALFA+ cohort by Benedet *et al.*,¹⁹ plasma GFAP enabled the separation of A+T- individuals from A-T- individuals ($P < 0.001$). However, plasma GFAP concentrations in A-T+ individuals were not significantly higher compared with the A-T- group, suggesting that plasma GFAP levels are more closely associated with astrogliosis and amyloid build-up than with tau pathology. Pereira *et al.*⁹ corroborated these findings using the Swedish BioFINDER-2 cohort ($n = 504$) with the same assay. Elevated plasma GFAP levels correlated with Aβ-PET outcomes when tau-PET was included as a covariate across cognitively impaired individuals ($P < 0.001$), Aβ-positive cognitively unimpaired individuals ($P = 0.007$) and Aβ-positive cognitively impaired individuals ($P = 0.041$). Conversely, no significant correlation was observed between GFAP levels and tau-PET when Aβ-PET was included as a covariate. These findings underscore a specific association between amyloid pathology and elevated GFAP levels, independent of tau pathology. GFAP expression appears to be primarily associated with Aβ plaques, as supported by existing literature.¹⁴⁵ Additionally, Rasing *et al.*¹⁴⁶ recently demonstrated that elevated circulating GFAP levels strongly correlate with early cerebral amyloid angiopathy. These findings reinforce the hypothesis that GFAP leakage is more closely associated with amyloid pathology in brain and vasculature than with tau pathology.

Blood GFAP level correlates with cognitive impairment severity

In addition to its correlation with amyloid pathology, plasma GFAP levels also gradually increase with the severity of

cognitive impairment across the Alzheimer's disease continuum, as reported by Chatterjee *et al.*²⁰ Their observations were based on the Australian Imaging, Biomarker and Lifestyle longitudinal cohort (AIBL, Australia) which included 181 individuals, with GFAP being quantified using the Quanterix Simoa Neurology-4-Plex Assay. Plasma GFAP was significantly higher in cognitively unimpaired A β -positive (CU- A β +), mild cognitively impaired A β -positive (MCI A β +) and Alzheimer's disease A β -positive (AD A β +) groups ($P < 0.0001$) compared with cognitively unimpaired A β -negative (CU A β -) and mild cognitively impaired A β -negative groups (MCI A β -) ($P < 0.0005$). This suggests that elevated blood GFAP levels are associated with amyloid burden in Alzheimer's disease, consistent with findings from other studies.^{9,19} Furthermore, GFAP levels also correlated with brain amyloid loading and disease severity, with significantly higher plasma GFAP in AD-A β + compared with MCI A β + ($P < 0.001$) and CU A β + ($P < 0.01$) as well as between MCI A β + against AD-A β + ($P < 0.001$). Over the course of a 36-month longitudinal analysis within the same cohort, GFAP levels continued to rise in MCI A β + and AD-A β + compared with controls, further supporting that plasma GFAP levels track with disease progression. This study was the first to demonstrate increased plasma GFAP levels in cognitively normal older adults at risk of Alzheimer's disease. Shen *et al.*²² further analysed a cross-sectional cohort of patients recruited from the Memory Clinic of the Huashan Hospital of Fudan University and the Chinese Alzheimer Biomarker and Lifestyle (CABLE) study (China, $n = 700$) using Quanterix single-molecule array single plex assay. Their findings as aligned with previous cohort studies, showing that plasma GFAP was incrementally increased along the course of Alzheimer's disease from preclinical and prodromal stages to Alzheimer's disease dementia ($P < 0.001$).

Blood GFAP is elevated in at-risk cognitively unimpaired individuals

Chatterjee *et al.*¹⁴⁷ had previously studied GFAP as a predictive biomarker for Alzheimer's disease in at-risk individuals without cognitive impairment. In this study, plasma GFAP levels were compared between cognitively normal older adults at risk of Alzheimer's disease with high PET-A β load (A β +) and those with low A β load (A β -) from the Kerr Anglican Retirement Village Initiative in Aging Health (KARVIAH) cohort ($n = 134$). A β + participants demonstrated significantly higher plasma GFAP concentrations compared with A β -, both before and after adjusting for known risk factors such as age, sex and APOE ϵ_4 status ($P < 0.0001$). These results suggest that elevated plasma GFAP levels could serve as an early indicator for predicting Alzheimer's disease development in at-risk individuals before the onset of cognitive decline. Through a longitudinal analysis within a cohort from the Shanghai Aging Study ($n = 118$), Shen *et al.*²² showed that cognitively normal individuals who later developed Alzheimer's disease presented higher baseline GFAP compared with those who did not

convert to Alzheimer's disease. The difference between converters and non-converters was significant ($P < 0.001$), with an AUC of 0.85. These findings support the utility of plasma GFAP as a predictive biomarker for Alzheimer's disease, even before the onset of cognitive decline.

Diagnosis perspectives of GFAP as a blood-based biomarker

Blood biomarkers: what they offer and linked challenges

CSF has historically been the preferred sample type for neurodegenerative disease research due to its proximity to the brain. However, its collection via lumbar puncture is invasive, and the volume that can be collected is limited. Imaging techniques such as PET scan, while effective, are costly, have low throughput and involve irradiation which reduces its large-scale implementation. Recently, significant efforts have been made to quantify brain-derived proteins in blood, offering a less invasive alternative. Blood sampling is advantageous due to lower costs and higher throughput. Nevertheless, the concentrations of brain-derived proteins in blood are extremely low compared with abundant blood proteins such as albumin and immunoglobulins. Consequently, blood-based biomarker testing requires highly sensitive measurement devices and meticulous pre-analytical handling to achieve the necessary performance standards.¹⁴⁸

In clinical practice, blood biomarkers hold promise for the diagnosis, prognosis and monitoring of Alzheimer's disease. These biomarkers offer several advantages, including reduced invasiveness, ease of sample collection, affordability, rapid implementation and broad acceptance. The accessibility of blood samples could also facilitate research into potential treatments, evaluation of therapeutic efficacy and a deeper understanding of the disease's pathological landscape, thereby serving as a promising strategy for screening. Despite the high accuracy of current clinically approved diagnosis and screening methods, such as amyloid and tau-PET imaging and CSF protein quantification, their limited availability, high cost and invasiveness urge the need for surrogate biomarkers for screening and diagnosis purposes.

Current and future directions: the ATNIVS framework

In accordance with The National Institute of Aging and Alzheimer's Association (NIA-AA) framework Alzheimer's disease is a biological construct that does not solely rely on clinical symptoms. Diagnosis involves identifying core pathological hallmarks and related biomarkers. The initial guidelines, published in 2011, have recently been updated.¹⁰ According to these guidelines, Alzheimer's disease biomarkers are categorized using the [AT(N)] classification system. 'A' refers to A β peptide deposition, identified through low levels of A β ₁₋₄₂, a decreased A β _{1-42/40} ratio and positive amyloid PET. 'T' represents the accumulation of abnormal pathologic tau protein in the brain, which is reflected in increased CSF concentration of phosphorylated tau and

It is increasingly recognized that Alzheimer's disease represents a spectrum of disorders with various genetic and potentially environmental risk factors that share similar and interrelated pathological and clinical features.¹⁴⁹

Potential application of GFAP in Alzheimer's disease diagnosis and disease monitoring

Plasma GFAP is increasingly recognized as a valuable biomarker that can be integrated into the existing diagnostic framework for Alzheimer's disease. While GFAP alone reflects astrocytic activation and neuroinflammation, which are common across various neurodegenerative diseases, its diagnostic utility is significantly enhanced when used alongside with established Alzheimer's disease biomarkers. Studies support the combined use of GFAP with other blood-based biomarkers to improve diagnostic accuracy, particularly in cases involving overlapping comorbidities, progressive neurodegeneration and amyloid accumulation.¹⁵⁰ Therefore biomarkers like NfL,¹⁵¹ associated with general neuronal damage, and Alzheimer's disease-specific biomarkers such as the $A\beta_{1-42}/A\beta_{1-40}$ ratio and pTau-181 have been explored in conjunction with GFAP.^{152,153} In a cohort with 252 subjects from Amsterdam Dementia Cohort, Verberk *et al.*¹⁵⁴ demonstrated that combining multiple biomarkers enhances diagnostic accuracy. Using the Simoa 4-Plex assay (Quanterix), they found that the ROC curve AUCs for predicting amyloid PET status were 0.71 for NfL, 0.81 for GFAP and 0.73 for the $A\beta_{1-42}/A\beta_{1-40}$ ratio. When these markers were combined, the AUC increased to 0.88. Similarly, Yang *et al.*¹⁵⁵ observed that combining pTau-181 with GFAP provided superior results for distinguishing amyloid status. With individual AUCs of 0.81 and 0.82, respectively, and a combined AUC of 0.86 in the Nevada Center for Neurodegeneration and Translational Neuroscience cohort (United States, $n = 144$). Among cognitively impaired participants ($n = 87$) the AUC further to 0.93, only marginal enhancement (0.01) upon addition of NfL. For differentiating frontotemporal dementia from cognitively unimpaired or non-neurodegenerative impairment, plasma NfL was quite accurate as corroborated by Sarto *et al.*¹⁵⁶ in a study involving 385 individuals from Alzheimer's disease and other cognitive disorders unit (Barcelona), with AUCs of 0.90 and 0.93. In this study, GFAP showed similar performances to pTau-181 in distinguishing Alzheimer's disease from CU ($P < 0.001$) and MCI ($P < 0.001$) individuals, with a strong correlation with $A\beta$ status. Additionally, GFAP both effectively distinguished Alzheimer's disease from other neurodegenerative dementias, such as dementia with Lewy bodies ($P < 0.001$). In contrast, total-tau did not significantly differentiate between these pathologies. Integrating GFAP into the existing ATN framework could enhance its ability to reflect the complex pathology of Alzheimer's disease, providing valuable insights into the role of reactive astrogliosis in Alzheimer's disease. Furthermore, apart from brain-related proteins, including additional biomarkers, into these analyses could improve patient care by addressing comorbid conditions like kidney dysfunction. This is particularly

important in elderly patients who might suffer from other chronic conditions that could impair Alzheimer's disease diagnosis.¹⁵⁷

Conclusion

Astrogliosis has emerged as a significant feature of Alzheimer's disease, characterized by the proliferation of structurally and functionally altered astrocytes. This pathological process is associated with the overexpression and release of GFAP in biofluids, which correlates with disease severity and progression. Plasma GFAP has demonstrated superior performance compared with its CSF counterpart in diagnosing Alzheimer's disease, particularly in distinguishing $A\beta$ -positive from $A\beta$ -negative individuals, providing prognostic information in at-risk populations and differentiating Alzheimer's disease from other neurodegenerative disorders such as frontotemporal dementia. These findings underscore the clinical relevance of GFAP as a biomarker for Alzheimer's disease-related astrogliosis.

Although the potential of targeting reactive astrocytes as a therapeutic strategy remains underexplored, they may represent promising candidates for therapies aimed at halting disease progression due to their early and prominent role in Alzheimer's pathology. However, while GFAP is a valuable biomarker, it is insufficient as a standalone marker in clinical practice. Incorporating GFAP into a panel of biomarkers enhances diagnostic accuracy for Alzheimer's disease by providing additional insights into astrocytic activation and neuroinflammation, which are critical aspects of the disease's pathology. This approach not only aids in early diagnosis and monitoring of disease progression but also helps differentiate Alzheimer's disease from other neurodegenerative conditions. By integrating GFAP within a multi-biomarker strategy, clinical practice can advance towards more precise and personalized management of Alzheimer's disease.

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Data availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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