

Glymphatic Dysfunction Coupled with Aberrant Distribution of Iron and Pathological proteins across AD Continuum

Zhen Yuan

zhengyuan@um.edu.mo

University of Macau <https://orcid.org/0000-0003-3061-6263>

Chantat Leong

University of Macau

Aocai Yang

Shandong Provincial Hospital Affiliated to Shandong First Medical University <https://orcid.org/0000-0003-0563-6959>

Ruisi Wang

University of Macau

Yu Sun

Department of Neurology, Xuanwu Hospital of Capital Medical University, Beijing, China, 100053

Jixin Luan

Department of Radiology, China-Japan Friendship Hospital, Beijing, China

Manxi Xu

Department of Radiology, China-Japan Friendship Hospital, Beijing, China

Yian Gao

Department of Radiology, China-Japan Friendship Hospital, Beijing 100029, China

Hongwei Yu

China-Japan Friendship Hospital

Yuli Wang

Department of Radiology, China-Japan Friendship Hospital

Ni Shu

State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research <https://orcid.org/0000-0003-2420-2910>

Bing Zhang

The Affiliated Drum Tower Hospital of Nanjing University Medical School

Guo-Lin Ma

Department of Radiology, China-Japan Friendship Hospital <https://orcid.org/0000-0002-4044-025X>

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Title Page

Glymphatic Dysfunction Coupled with Aberrant Distribution of Iron and Pathological proteins across AD Continuum

Chantat Leong^{1,2#}, Aocai Yang^{3#}, Ruisi Wang¹, Yu Sun⁴, Jixin Luan⁵, Manxi Xu⁶, Yian Gao⁶, Hongwei Yu⁶, Yuli Wang⁶, Ni Shu⁷, Bing Zhang⁸, Guolin Ma^{6*}, Zhen Yuan^{1,2*}

¹Centre for Cognitive and Brain Sciences, University of Macau, Macau SAR 999078, China

²Faculty of Health Sciences, University of Macau, Macau SAR 999078, China

³Department of Radiology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, China

⁴Department of Neurology, China-Japan Friendship Hospital, Beijing 100029, China

⁵Department of Radiology, Qilu Hospital of Shandong University, Jinan 250012, Shandong, China

⁶Department of Radiology, China-Japan Friendship Hospital, Beijing 100029, China

⁷State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing 100875, China

⁸Department of Radiology, the affiliated Drum tower hospital of Nanjing University medical school, Beijing, China

* Corresponding author:

Prof. Zhen Yuan

Address: Centre for cognitive and brain sciences, University of Macau, Macau SAR 999078, China.

Email: zhenyuan@um.edu.mo

Prof. Guolin Ma

Address: Department of Radiology, China-Japan Friendship Hospital, No. 2 East Yinghua Road, Chaoyang District, Beijing 100029, China.

Email: maguolin1007@qq.com,

These authors contributed equally to this article.

Abstract

Alzheimer's disease (AD) involves impaired glymphatic clearance and altered iron-related and macromolecular tissue composition, yet how these processes are interconnected across the disease continuum remains unclear. We developed a multimodal MRI framework integrating complementary glymphatic indices with paramagnetic (χ_{para}) and diamagnetic ($|\chi_{\text{dia}}|$) susceptibility measures across AD continuum. Multivariate partial least squares analysis identified cross-modal covariance patterns, followed by disease-stage analyses and pseudotime modeling to characterize their stage-related and pseudotemporal organization. Glymphatic and susceptibility measures showed coordinated associations at both regional and network levels, with prominent involvement of temporal, limbic, and subcortical regions. Disease-stage-related relationships were predominantly characterized by perivascular space (PVS)- and basal forebrain (BF)-related coupling with χ_{para} and $|\chi_{\text{dia}}|$ in temporal and limbic regions, whereas network-level alterations were primarily associated with PVS. Pseudotemporal modeling revealed heterogeneous organization, with PVS-related regional patterns predominating earlier, BF-related regional patterns at intermediate positions, and selective network-level involvement later. These findings suggest that glymphatic dysfunction and altered tissue composition represent interconnected components of disrupted brain fluid-tissue homeostasis across the AD continuum.

Keywords

Alzheimer's disease, Cerebrospinal fluid flow; Functional MRI; Quantitative susceptibility mapping; Glymphatic function; Iron accumulation

1. Introduction

Alzheimer's disease (AD) is characterized by progressive neurodegeneration arising from impaired clearance of metabolic waste, including amyloid- β (A β), tau, and other neurotoxic molecules. Inefficient removal of these proteins exacerbates their aggregation, amplifies oxidative and metabolic stress, and disrupts neuronal function, ultimately accelerating neurodegeneration. Converging evidence suggests that failure of brain fluid clearance systems, encompassing neurovascular coupling, blood-brain barrier (BBB) permeability, and glymphatic transport, plays a central mechanistic role in this process [1-4]. Dysregulated cerebrospinal fluid (CSF) transport is increasingly recognized as a critical component of impaired clearance in AD, and its disruption is expected not only to compromise solute removal but also to promote the accumulation of neurotoxic substances.

The glymphatic system facilitates CSF-interstitial fluid exchange along perivascular pathways regulated by astrocytic aquaporin-4 (AQP4) and represents a key mechanism for metabolic waste clearance in the brain. AQP4 mislocalization, reduced CSF influx, and impaired perivascular solute transport are observed, leading to diminished clearance of A β and tau and promoting toxic protein accumulation [5-7]. Previous studies further show that AQP4 depolarization is associated with both amyloid burden and cognitive impairment, while genetic variations affecting AQP4 expression modulate AD risk and cognitive trajectories [8, 9]. Advances in magnetic resonance imaging (MRI) enable *in vivo* quantification of multiple, complementary aspects of glymphatic and perivascular function. These MRI-derived markers capture complementary physiological aspects of glymphatic and perivascular function rather than a single process. Functional measures, including BOLD-CSF coupling, primarily reflect neurovascular-driven CSF inflow dynamics. Diffusion-derived indices, such as the DTI-ALPS index and free-water (FW), characterize water diffusivity along perivascular pathways and extracellular fluid mobility. Structural markers, including perivascular space (PVS), choroid plexus (CP), cerebrospinal fluid (CSF), characterize anatomical compartments involved in perivascular transport, CSF production, and CSF circulation [10-15]. Basal forebrain (BF) volume was included to reflect the cholinergic regulation of glymphatic function, as cholinergic neurons in BF modulate cerebral vascular dynamics and CSF flow [16].

Previous studies across the AD spectrum have reported reduced BOLD-CSF coupling and lower DTI-ALPS indices, consistent with impaired neurovascularly mediated CSF inflow and reduced perivascular transport efficiency [11, 13, 14]. Increased FW and PVS reflect extracellular fluid accumulation and impaired interstitial drainage [14, 15], whereas enlargement of the CP and ventricular CSF compartments has been associated with altered CSF production, barrier function, and fluid turnover [7, 15]. Degeneration of the cholinergic basal forebrain may further weaken the vascular and CSF dynamics that support glymphatic transport [5, 6, 16]. Collectively, these abnormalities suggest that AD-related glymphatic dysfunction involves multiple components of brain fluid transport and clearance, including CSF dynamics, perivascular transport, interstitial fluid mobility, and the structural properties of fluid-transport pathways. These processes are

physiologically interconnected and jointly support fluid transport and solute clearance from brain tissue. Integrating these complementary glymphatic measures may therefore provide a broader representation of glymphatic dysfunction by capturing coordinated alterations across multiple interconnected clearance-related processes in AD.

While glymphatic dysfunction is commonly considered a failure of A β and tau clearance, impaired CSF-interstitial fluid exchange is expected to influence the broader microstructural environment of brain tissue. Beyond the accumulation of soluble proteins, reduced clearance efficiency may promote iron retention and the accumulation of neurotoxic macromolecules, contributing to microstructural alterations in vulnerable regions [17-19]. Consistent with this view, iron homeostasis is profoundly altered in AD. Aging-related shifts in iron regulation, together with microglial activation and lysosomal dysfunction, promote iron retention in vulnerable cortical and subcortical regions. Excess iron catalyzes reactive oxygen species production, accelerates A β aggregation and tau phosphorylation, and contributes to mitochondrial damage and cellular death [20-23]. Previous studies also found that iron accumulation co-localizes with A β and tau aggregation, suggesting that altered tissue composition may emerge as a downstream consequence of impaired clearance-related processes [24].

Quantitative susceptibility mapping (QSM) provides a noninvasive approach to characterize microstructural tissue composition by capturing magnetic susceptibility signals sensitive to iron, myelin, and other macromolecular constituents. Conventional QSM reflects a composite voxel-wise signal in which paramagnetic and diamagnetic contributions may partially cancel each other, limiting biological specificity. By decomposing QSM into paramagnetic (χ_{para}) and diamagnetic (χ_{dia}) components, it is possible to more precisely distinguish susceptibility contributions arising from different tissue constituents. χ_{para} is predominantly sensitive to paramagnetic sources such as iron, whereas χ_{dia} reflects diamagnetic contributions from myelin, proteins, and other macromolecular tissue constituents [25-28]. Previous QSM studies consistently have demonstrated regional susceptibility alterations associated with iron-related tissue changes across the AD spectrum, and vascular dysfunction, blood-brain barrier breakdown, and impaired interstitial drainage have been implicated in abnormal iron accumulation [29, 30]. Experimental evidence further suggests the link between glymphatic dysfunction and iron retention, with impaired glymphatic clearance accompanied by increased tissue iron deposition and experimental inhibition of glymphatic function further exacerbating iron accumulation [31]. Human MRI studies have also reported associations between glymphatic measures and regional QSM-derived iron deposition [32]. Together, these findings provide a biological basis for a relationship between glymphatic dysfunction and susceptibility-related tissue alterations. However, existing evidence has largely focused on individual glymphatic measures or specific brain regions, leaving unclear how multiple glymphatic-related processes and susceptibility alterations covary across the human AD continuum. In particular, whether these cross-modal relationships persist beyond their shared associations with disease stage and how their expression changes across disease stages remain poorly understood.

We hypothesized that glymphatic and QSM measures would exhibit cross-modal associations. Furthermore, the expression of these cross-modal associations would become more extensive across the AD continuum, involving a broader range of glymphatic-related processes and susceptibility alterations across increasingly distributed brain regions. To test these hypotheses, we developed a multimodal MRI framework integrating complementary glymphatic indices with paramagnetic and diamagnetic susceptibility components. We first used multivariate analysis to identify cross-modal relationships between glymphatic and susceptibility measures. We then used pairwise correlation analyses to localize these relationships across specific glymphatic indices and brain regions and to examine how their extent differed across disease stages. Finally, pseudotime modeling was used to characterize how the identified cross-modal features changed along the AD continuum. (Fig. 1).

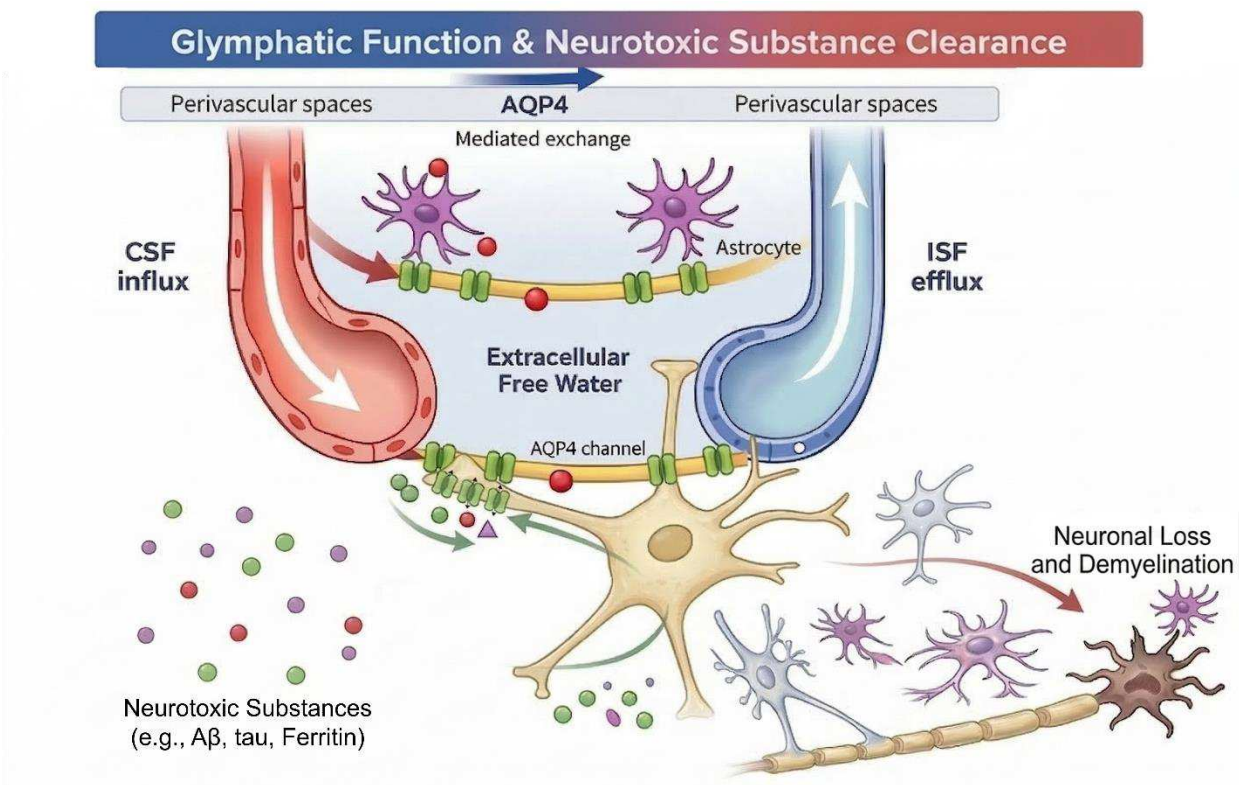


Fig. 1. Schematic of a conceptual model illustrating how glymphatic dysfunction may contribute to iron accumulation and other macromolecular neurotoxic tissue composition through impaired CSF-ISF exchange along perivascular pathways in Alzheimer's disease, and eventually cause the neuron loss and demyelination.

2. Results

2.1 Clinical characteristics

The clinical characteristics of this cohort are shown in Table 1. The age, sex, education level, and cognitive scores differed across the AD continuum.

148 **Table 1. Demographic and clinical characteristics of AD, MCI, SCD and HC groups**

Characteristics	AD	MCI	SCD	HC	<i>F</i> -value/ χ^2	<i>p</i> -value	effect size
Age	71.83±9.02 (N = 178)	66.74±8.02 (N = 250)	65.69±7.92 (N = 154)	64.21±8.71 (N = 256)	30.28	<0.0001	0.098
Sex(M/F)	(109/69) (N = 178)	(177/73) (N = 250)	(120/34) (N = 154)	(166/90) (N = 256)	12.81	<0.05	0.124
Education (years)	10.53±4.29 (N = 178)	10.73±3.42 (N = 250)	12.29±3.63 (N = 154)	11.23±4.47 (N = 254)	6.54	<0.05	0.023
MMSE	19.01±5.33 (N = 178)	27.18±2.41 (N = 250)	28.18±1.79 (N = 154)	28.33±1.72 (N = 256)	380.05	<0.001	0.59
MoCA	12.06±5.8 (N = 67)	21.37±3.97 (N = 83)	24.2±2.86 (N = 102)	25.09±2.93 (N = 89)	166.51	<0.001	0.6
HAMA	9.76±7.71 (N = 55)	5.33±5.85 (N = 81)	6.5±5.68 (N = 101)	3.5±6.37 (N = 66)	11.31	<0.001	0.1
HAMD	5.11±4.55 (N = 55)	3.89±4.76 (N = 81)	5.64±5.15 (N = 101)	2.68±3.28 (N = 66)	6.3	<0.05	0.059
RAVLT	6.28±12.13 (N = 71)	8.99±6.6 (N = 81)	12.13±7.16 (N = 101)	15.88±5.57 (N = 66)	18.03	<0.001	0.15
CDT	13.62±10.29 (N = 55)	23.32±6.51 (N = 81)	25.82±4.51 (N = 101)	27.5±2.3 (N = 66)	60.43	<0.001	0.38
BNT	16.04±6.16 (N = 71)	23.81±4.38 (N = 81)	26.16±3.47 (N = 101)	27.29±2.47 (N = 66)	101.83	<0.001	0.49
VFT	21.91±8.48 (N = 53)	29.23±6.31 (N = 30)	40.85±9.35 (N = 39)	48.33±7.91 (N = 12)	57.57	<0.001	0.57

149 Note: Data is presented as mean ± standard deviations (SD). MMSE: Mini-Mental State Examination, MoCA:
150 Montreal Cognitive Assessment test, HAMD: Hamilton depression rating, HAMA: Hamilton anxiety rating, RAVLT:
151 Rey Auditory Verbal Learning Test, CDT: Clock Drawing Test, BNT: Boston Naming Test, VFT: Verbal Fluency
152 Test.

153 2.2 Group differences in glymphatic and susceptibility measures

154 Eight glymphatic-related measures demonstrated significant overall differences across
155 diagnostic groups (Fig. 2A; Supplementary Table S1), including DTI-ALPS ($F = 28.13, p < 0.001$),
156 BF ($F = 8.79, p < 0.001$), CSF ($F = 19.33, p < 0.001$), FW ($F = 31.55, p < 0.001$), CP ($F = 15.96,$
157 $p < 0.001$), gBOLD-CSF coupling ($F = 3.44, p = 0.016$), tBOLD-CSF coupling ($F = 2.71, p =$
158 0.044), and PVS ($F = 6.74, p < .001$). The most pronounced differences were observed in the AD
159 group. Compared with HC, SCD, and MCI, AD showed lower DTI-ALPS and BF values and
160 higher CSF, FW, and CP measures (all FDR-corrected $p < 0.001$). BOLD-CSF coupling also
161 differed across groups, with weaker gBOLD-CSF coupling in AD than HC and MCI and weaker
162 tBOLD-CSF coupling in AD than MCI (FDR-adjusted $p < 0.05$). PVS showed higher values in
163 SCD and MCI than HC but lower values in AD than MCI (FDR- corrected $p < 0.05$).

164 Both χ_{para} and $|\chi_{\text{dia}}|$ showed significant group differences across multiple cortical and
165 subcortical regions (Fig. 2B; Supplementary Table S2). Significant $|\chi_{\text{dia}}|$ differences were observed
166 in the OG ($F = 14.79, p < 0.001$), ITG ($F = 5.78, p < 0.001$), and DN ($F = 7.37, p < 0.001$).
167 Significant χ_{para} differences were observed in the OG ($F = 9.61, p < 0.001$), STG ($F = 8.88, p <$
168 0.001), ITG ($F = 6.41, p < 0.001$), FuG ($F = 5.67, p < 0.001$), PhG ($F = 11.37, p < 0.001$), and

Pcun ($F = 7.83, p < 0.001$). Post-hoc comparisons showed that AD had lower $|\chi_{\text{dia}}|$ values than both SCD and MCI in the OG and ITG, whereas higher $|\chi_{\text{dia}}|$ values were observed in the DN, with AD showing higher values than SCD and MCI (FDR-adjusted $p < 0.05$). For χ_{para} , AD showed lower values than SCD and/or MCI in the OG, STG, ITG, FuG, and PhG, whereas AD showed higher values than MCI in the Pcun (FDR-adjusted $p < 0.05$).

At the network level, significant $|\chi_{\text{dia}}|$ differences were observed in the LIM ($F = 13.13, p < 0.001$) and FPN ($F = 7.83, p < 0.001$), whereas significant χ_{para} differences were observed in the LIM ($F = 12.19, p < 0.001$) and DMN ($F = 10.57, p < 0.001$). Post-hoc comparisons showed lower $|\chi_{\text{dia}}|$ values in AD than both SCD and MCI in the LIM and FPN. Similarly, AD showed lower χ_{para} values than both SCD and MCI in the LIM and DMN (FDR-adjusted $p < .05$). Sensitivity analyses additionally adjusting for GMV showed that the susceptibility differences remained significantly differences, with additional ROIs and networks reaching significance (Fig. 2B, Supplementary Table S3).

2.3 Multivariate glymphatic-susceptibility components identified by PLS

LV1 captured the dominant cross-modal covariance pattern, explaining 54-56% of the covariance at the ROI level and 62–63% at the network level ($|\chi_{\text{dia}}|$: ROI 0.538, NET 0.633; χ_{para} : ROI 0.555, NET 0.616). Subsequent latent variables each explained substantially smaller proportions of covariance (2-34%) and were therefore not examined further (Supplementary Table S4).

At the ROI level, LV1 showed stable susceptibility contributions ($|\text{BR}| > 5$) predominantly in temporal, limbic, and subcortical regions, with all stable QSM features exhibiting negative BRs (Fig. 2B). For $|\chi_{\text{dia}}|$, stable negative contributions were observed in the OG, FuG, PhG, ITG, MTG, Put, BG, CG, Hipp, and Amyg, whereas the corresponding glymphatic pattern was characterized by stable negative contributions from PVS and BF. For χ_{para} , stable negative susceptibility contributions were observed in the FuG, OG, PhG, MTG, ITG, STG, CG, Hipp, and Psts. On the glymphatic side, FW showed a stable positive contribution, whereas BF, PVS, and DTI-ALPS showed stable negative contributions.

At the network level, LV1 showed a distinct pattern in which all stable susceptibility features exhibited positive BRs (Fig. 2B). For $|\chi_{\text{dia}}|$, stable positive contributions were observed in the FPN, DMN, VAN, and DAN, accompanied by a stable positive contribution from PVS and a stable negative contribution from gBOLD-CSF coupling. For χ_{para} , stable positive susceptibility contributions were observed in the VIS, and DMN, with the same glymphatic pattern of a positive PVS contribution and a negative gBOLD-CSF coupling contribution (Fig. 2C).

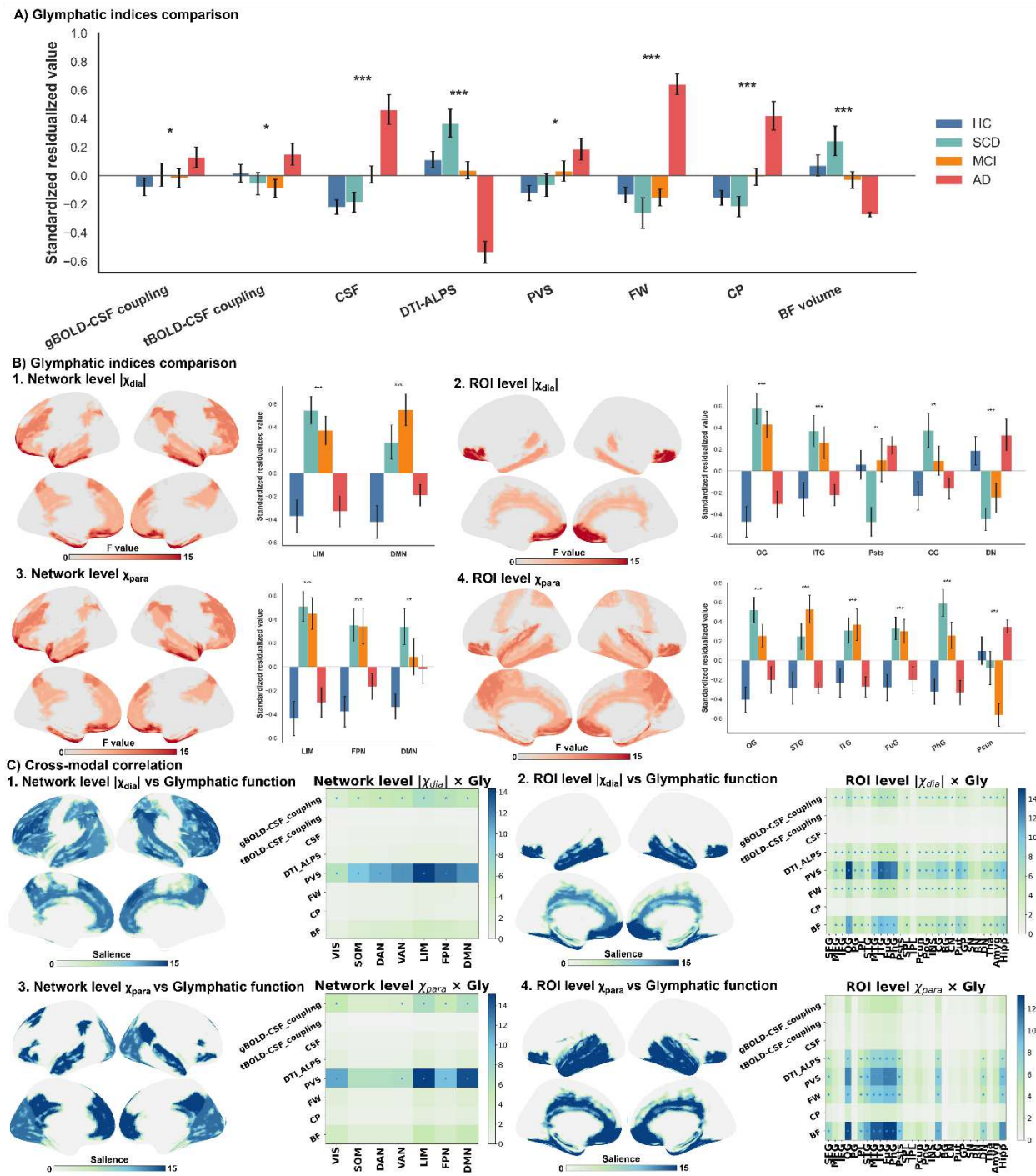


Fig. 2. Univariate comparison and cross-modal associations between glymphatic function and magnetic susceptibility. (A) Group comparisons of glymphatic-related indices across diagnostic groups (HC, SCD, MCI, and AD). Bars represent standardized residualized values after adjustment for age, sex, and scanner, with error bars indicating standard errors. Asterisks indicate significant overall group effects after FDR correction. (B) Group comparisons of $|\chi_{dia}|$ and χ_{para} at the network and ROI levels. Surface maps show the spatial distribution of F values, and bar plots show standardized residualized values after adjustment for age, sex, scanner, and diagnostic groups. Asterisks indicate significant overall group effects after FDR correction (* $q < 0.05$, ** $q < 0.01$, and *** $q < 0.001$).

(C) Multivariate PLS results for the dominant latent variable (LV1) at both network and ROI levels for $|\chi_{\text{dia}}|$ and χ_{para} . Surface maps show the spatial distribution of LV1 loadings, and bar plots indicate the strongest glymphatic and susceptibility contributors. Asterisks mark features with significant bootstrap ratios ($|\text{BR}| \geq 5$). Abbreviations: PLS, partial least squares; LV, latent variable; BR, bootstrap ratio; FDR, false discovery rate.

2.4 Stage-specific patterns of glymphatic-susceptibility associations across the AD continuum

Stage-specific correlation analyses were performed using stable glymphatic and susceptibility features identified from the PLS models ($|\text{BR}| > 5$). At the ROI level, stage-related associations were predominantly characterized by BF- and PVS-related cross-modal pairs involving temporal, occipital, and limbic regions. For $|\chi_{\text{dia}}|$, BF-related pair scores showed negative stage trends with susceptibility measures in the FuG, OG, MTG, ITG, Hipp, and PhG (Spearman's $\rho = -0.351$ to -0.314 ; $\eta^2 = 0.066$ - 0.099 , FDR-corrected $p < 0.001$), whereas PVS-related pair scores showed negative stage trends in the FuG, MTG, ITG, Hipp, OG, BG, and PhG ($\rho = -0.325$ to -0.261 ; $\eta^2 = 0.065$ - 0.103 , FDR-corrected $p < 0.001$). For χ_{para} , 12 pairs met both criteria. BF-related pair scores showed negative stage trends in the FuG, OG, PhG, ITG, and MTG ($\rho = -0.356$ to -0.336 ; $\eta^2 = 0.081$ - 0.107 , FDR-corrected $p < 0.001$), but a positive stage trend in the PL ($\rho = 0.261$; $\eta^2 = 0.105$, FDR-corrected $p < 0.001$). PVS-related pair scores similarly showed negative stage trends in the MTG, FuG, ITG, OG, and PhG ($\rho = -0.303$ to -0.265 ; $\eta^2 = 0.066$ - 0.096 , FDR-corrected $p < 0.001$), while the PVS-PL pair showed a positive stage trend ($\rho = 0.291$; $\eta^2 = 0.072$, FDR-corrected $p < 0.001$).

At the network level, PVS-related pair scores showed positive stage trends with the SOM ($\rho = 0.320$, $\eta^2 = 0.092$), DAN ($\rho = 0.272$, $\eta^2 = 0.082$, FDR-corrected $p < 0.001$), and VAN ($\rho = 0.245$, $\eta^2 = 0.064$, FDR-corrected $p < 0.001$) in $|\chi_{\text{dia}}|$. For χ_{para} , only the PVS-LIM pair showed a negative stage trend ($\rho = -0.280$, $\eta^2 = 0.084$, FDR-corrected $p < 0.001$).

Together, these findings indicate that stage-specific patterns of glymphatic-susceptibility associations were concentrated primarily at the ROI level, particularly in BF- and PVS-related associations with temporal, occipital, and limbic susceptibility measures (Fig. 3A-B).

2.5 Pseudotemporal organization of glymphatic-susceptibility alterations

DPM identified distinct early-, intermediate-, and late-altering patterns among the 29 stage-related cross-modal features. Thirteen features showed pronounced and well-defined pseudotemporal trajectories, including 6 early-, 6 intermediate-, and 1 late-altering pattern.

The early-altering pattern was characterized exclusively by PVS-related ROI associations, including PVS $\times$ FuG, MTG, and PhG for $|\chi_{\text{dia}}|$ and PVS $\times$ MTG, OG, and ITG for χ_{para} . The intermediate-altering pattern was dominated by BF-related ROI associations, including BF $\times$ FuG, PhG, and MTG for $|\chi_{\text{dia}}|$ and BF $\times$ FuG, OG, and PhG for χ_{para} . The late-altering pattern was represented by the network-level PVS $\times$ SOM association for $|\chi_{\text{dia}}|$. Collectively, these representative trajectories demonstrated distinct pseudotemporal organization among the stage-related cross-modal features, with PVS-related regional patterns predominating at earlier

pseudotime positions, BF-related regional patterns at intermediate positions, and a PVS-related somatomotor network pattern at the later pseudotime position (Fig. 3C).

3.5 Associations between glymphatic-susceptibility patterns and cognition

Four ROI-level $|\chi_{dia}|$ features showed nominal indirect associations between disease stage and MMSE, predominantly involving PVS-related patterns in the MTG (bootstrap $p = 0.009$), Hipp ($p = 0.012$), and FuG ($p = 0.028$), together with a BF-MTG pattern ($p = 0.022$). None of these indirect effects survived FDR correction across the 29 cross-modal features (FDR-corrected $p = 0.180$ -0.203).

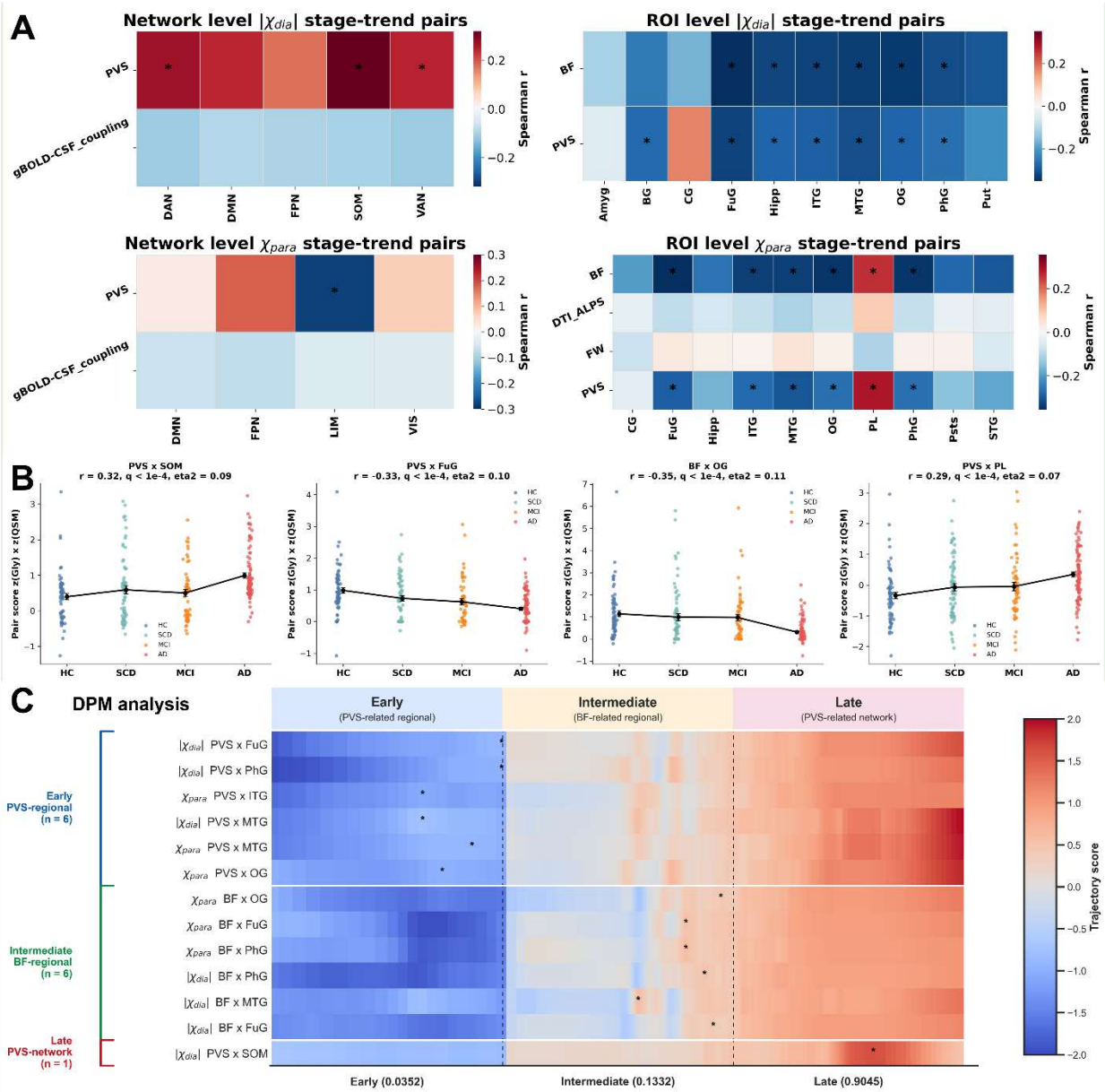


Fig. 3. Stage-specific analysis and disease progression modeling of glymphatic-susceptibility pairs. (A) Stage-related glymphatic – susceptibility pairs from significant PLS components, Pair-derived cross-modal scores were

calculated as the product of standardized glymphatic and susceptibility measures and examined for associations with disease stage (HC → SCD → MCI → AD). Colors represent spearman correlation between each cross-modal pair score and disease stage. Stars denote glymphatic-susceptibility pairs that were significant with FDR correction and the $\eta^2 > 0.06$. (B) Representative scatter plots illustrating the relationships between selected glymphatic-susceptibility pairs. Regression lines indicate overall stage-related trends. (C) Thirteen representative cross-modal features are shown according to their pseudotemporal trajectories. Rows represent normalized trajectories of individual pair-derived cross-modal features across pseudotime (0-1). Vertical dashed lines indicate the boundaries between the early, intermediate, and late pseudotemporal intervals, and asterisks indicate the estimated characteristic position of each trajectory along pseudotime. Colors represent normalized trajectory scores. Abbreviations: DPM, disease progression modeling; χ_{para} , paramagnetic susceptibility; $|\chi_{\text{dia}}|$, absolute diamagnetic susceptibility.

4. Discussion

The present findings identified coordinated cross-modal relationships between glymphatic function and susceptibility-derived tissue properties across the AD continuum. These two imaging domains exhibited a cross-modal covariance structure involving multiple aspects of glymphatic function, including perivascular transport, interstitial fluid mobility, and neurovascular-driven CSF dynamics, that persisted beyond their common associations with disease stage and was preferentially expressed in AD-vulnerable temporal, limbic, and subcortical regions. Within this broader covariance structure, disease-stage-related alterations were concentrated in PVS- and BF-related relationships with susceptibility in temporal, and limbic regions and showed heterogeneous pseudotemporal organization, with PVS-related regional patterns predominating at earlier positions and BF-related regional patterns at intermediate positions. Together, these findings provide a multimodal perspective on the coordinated alterations in brain clearance-related function and tissue composition in AD, offering a unified lens through which regional vulnerability and disease progression.

Glymphatic alterations were widespread across the AD continuum, with the most pronounced abnormalities observed in AD. Reductions in BOLD-CSF coupling, BF, and ALPS efficiency, together with elevated FW, and CSF and CP measures, point to disruption of CSF-related compartments, neurovascular-driven CSF dynamics [11, 12], perivascular diffusion, and interstitial fluid mobility [7, 13, 15]. PVS showed a distinct pattern, with higher values in SCD and MCI than HC, but lower values in AD than MCI, suggesting that structural changes in perivascular spaces may vary across disease stages rather than increase uniformly with disease severity. These heterogeneous trajectories across these measures suggest that different components of glymphatic function may be differentially affected across the AD continuum. In parallel, the χ_{para} values were reduced in the temporal, parietal, and subcortical cortical regions, including the OG, STG, ITG, FuG, and PhG, whereas the Pcun showed a different pattern, with higher values in AD than MCI. Increased χ_{para} has been widely interpreted as reflecting iron accumulation [28-30]. However, χ_{para} reductions in our cohort should not be interpreted as contradictory. Rather, it is compatible with a dynamic and region-dependent model of iron remodeling in AD. The cortical reductions in χ_{para} are consistent with prior evidence of regionally selective iron redistribution in

AD [33], a process that has been linked to mitochondrial stress and dysregulated cellular iron handling [34, 35], reflecting impaired iron storage, reduced local iron demand and transport capacity in neurodegenerative progress.

The $|\chi_{\text{dia}}|$ findings demonstrated alterations in diamagnetic tissue composition in the OG, ITG, and DN, with lower cortical values but higher values in the DN in AD relative to SCD and MCI. A recent study indicated plastic myelination in the deep cortical layer in older adults, accompanied by age-related cortical thinning [36]. Additionally, $|\chi_{\text{dia}}|$ has been shown to reflect macromolecular, structural composition, and calcium-related components beyond myelin content [25-27, 35, 37], and reductions in $|\chi_{\text{dia}}|$ are consistent with alterations in protein density, cellular integrity, and extracellular organization accompanying neurodegeneration [28]. The opposing regional patterns of χ_{para} and $|\chi_{\text{dia}}|$ may represent spatially heterogeneous disturbances in iron homeostasis and tissue constituents during neurodegeneration. At a broader spatial scale, the involvement of the LIM and FPN for $|\chi_{\text{dia}}|$ and the LIM and DMN for χ_{para} further indicates that susceptibility alterations are organized not only regionally but also across large-scale cortical systems. Importantly, both χ_{para} and $|\chi_{\text{dia}}|$ differences remained significant after adjustment for GM, indicating that these alterations cannot be fully attributed to regional atrophy or volume effects. These findings demonstrate substantial but heterogeneous alterations in both glymphatic function and susceptibility tissue properties across the AD continuum, raising the possibility that these two sets of abnormalities may be systematically related rather than simply occurring in parallel.

Beyond univariate alterations, the cross-modal analyses revealed that glymphatic and susceptibility features were not independently distributed but exhibited systematic cross-modal covariance. At the regional level, stable susceptibility contributions were concentrated in temporal, limbic, and subcortical regions, accompanied by glymphatic features involving PVS, BF, FW, and DTI-ALPS. The involvement of multiple glymphatic features suggests that susceptibility-derived tissue properties are related not to a single aspect of glymphatic function, but reflects coordinated relationships between perivascular pathway integrity, interstitial fluid mobility, and large-scale solute exchange, linking microstructural tissue composition with fluid transport dynamics [17, 19]. The glymphatic pattern differed between the two susceptibility components: $|\chi_{\text{dia}}|$ covariance was primarily associated with PVS and BF, whereas χ_{para} covariance additionally involved FW and DTI-ALPS. This distinction may indicate that paramagnetic and diamagnetic tissue properties have partially different relationships with the physiological and structural components of brain fluid transport. Notably, this coupling was preferentially expressed in regions such as FuG, BG, CG, and Hipp, areas characterized by high metabolic demand, dense vascular interfaces, and early AD vulnerability [38, 39], indicates that coupling between clearance-related and tissue compositional features is most prominent within circuits that rely heavily on efficient fluid transport and perivascular regulation.

At the network level, stable glymphatic and susceptibility contributions further indicated that this cross-modal relationship was organized across large-scale cortical systems, involving the LIM, DMN, and FPN. PVS and gBOLD-CSF coupling contributed consistently to the network-level

covariance, suggesting that both perivascular structural characteristics and neurovascular-driven CSF dynamics are related to the broader cortical organization of susceptibility tissue properties [11, 40, 41]. The convergence of these two spatially distributed processes suggests that glymphatic-related physiology and tissue susceptibility may be coordinated not only locally but also at the level of large-scale cortical organization. Together, these patterns may reflect an interconnected relationship between glymphatic function and tissue composition, in which fluid transport and exchange shape the distribution and turnover of tissue constituents, while the local tissue environment may in turn influence fluid movement and exchange. Such glymphatic-tissue composition coupling may therefore operate across both regional and large-scale cortical systems.

Across the AD continuum, stage-related changes in glymphatic–tissue composition coupling were predominantly expressed at the regional level [2]. These relationships were largely characterized by PVS- and BF-related indices with susceptibility measures in temporal and limbic regions, with considerable overlap between $|\chi_{\text{dia}}|$ and χ_{para} . The recurrence of these regional relationships across both susceptibility components suggests that AD-related changes may preferentially involve anatomical sites where perivascular and cholinergic regulation intersect with local tissue composition [16, 42]. This regional vulnerability suggests that AD progression may increasingly disrupt the interaction between solute exchange and tissue composition in temporal and limbic regions, with alterations in perivascular and cholinergic regulation potentially contributing to the local redistribution of iron and other microstructural tissue composition. Stage-related glymphatic–tissue composition changes were also evident across large-scale cortical systems, with PVS-related susceptibility relationships involving the SOM, DAN, VAN, and LIM [43, 44]. These findings suggest a progressive alteration of glymphatic–tissue composition coupling across the AD continuum, with the consistent involvement of PVS highlighting a prominent contribution of perivascular pathways to network-level alterations.

Pseudotemporal analysis further showed that these stage-related relationships were not organized synchronously across the AD continuum. Earlier pseudotime positions were characterized predominantly by PVS-related coupling with regional susceptibility measures, particularly in temporal regions, whereas intermediate positions were dominated by BF-related regional relationships. A PVS– $|\chi_{\text{dia}}|$ relationship at the SOM network level emerged at a later pseudotime position. This pseudotemporal organization showed that alterations in glymphatic-tissue composition coupling may evolve across anatomical scales, from regional relationships at earlier and intermediate positions to large-scale network at later positions. These findings suggest that AD does not affect glymphatic-susceptibility coupling features uniformly but progressively disrupts the organization of the clearance-tissue composition system according to regional dependence on fluid transport and metabolic support. Temporal regions rely heavily on efficient CSF-interstitial exchange to maintain local tissue homeostasis, appear particularly vulnerable to early disruption. As disease progresses, this disruption extends into large scale network, reflecting a hierarchical breakdown of the physiological coupling between clearance efficiency and tissue composition. This spatial gradient therefore reflects not merely where abnormalities occur but also

characterizes the spatiotemporal evolution of both clearance dysfunction and neurotoxic or tissue compositional burden.

These findings suggest that glymphatic function and susceptibility-derived tissue composition constitute an interconnected tissue-clearance pattern, in which fluid transport and exchange are closely coupled with the distribution and organization of tissue constituents. Within this system, coordinated alterations in specific glymphatic and tissue compositional features may reflect progressive disruption of the brain clearance environment across the AD continuum, potentially through impaired solute exchange that favors iron retention and macromolecular alterations, or through broader pathological processes that simultaneously compromise clearance function and tissue integrity [6, 38, 45]. The pseudotemporal organization further suggests that this disruption may evolve across anatomical scales, from predominantly regional alterations at earlier and intermediate positions toward selective large-scale network involvement at later positions. Although these stage-related cross-modal alterations did not significantly mediate cognitive performance, their spatial and pseudotemporal organization provides a systems-level view of how glymphatic and tissue compositional abnormalities may become progressively coordinated across the AD continuum. The cross-modal framework may also provide a useful basis for exploring physiological mechanisms underlying neurodegenerative processes and for developing imaging markers sensitive to system-level vulnerability.

Several limitations should be noted. First, the cross-sectional design precludes causal inference and prevents direct characterization of disease trajectories; longitudinal studies integrating MRI with fluid or PET biomarkers will be necessary to clarify the temporal ordering of clearance and susceptibility changes. Second, the glymphatic and susceptibility indices used here serve as indirect proxies, each capturing only part of the underlying biology and potentially influenced by vascular, inflammatory, or metabolic factors not assessed in this study. Third, the DPM analysis was based on cross-sectional data and therefore represents a simulated pseudotemporal ordering rather than directly observed temporal changes. Longitudinal studies are needed to determine whether the identified regional-to-network pattern reflects their actual temporal evolution. Finally, applying this framework to additional neurodegenerative or cerebrovascular conditions may help determine whether the cross-modal architecture is AD-specific or reflects a broader clearance–accumulation motif. Despite these constraints, the present multimodal approach provides a scalable foundation for integrating fluid-transport and susceptibility-based markers toward more comprehensive imaging indicators of neurodegenerative vulnerability.

This study provides evidence that glymphatic dysfunction and susceptibility-derived tissue alterations in AD are components of an interconnected physiological process rather than parallel abnormalities. By integrating complementary measures of brain fluid transport with paramagnetic and diamagnetic susceptibility, our findings suggest that AD-related disruption of solute exchange is closely linked to alterations in iron-related and microstructural tissue composition across the disease continuum. These alterations may evolve across anatomical scales, from predominantly

regional relationships to large-scale network involvement. This tissue–clearance composition perspective extends the conventional view of glymphatic dysfunction beyond impaired protein clearance and provides a systems-level framework for understanding how disruption of brain fluid homeostasis may accompany broader changes in the tissue microenvironment during neurodegeneration.

4. Methods

To examine the relationships between glymphatic function and microstructural susceptibility, a multimodal MRI framework was used to integrate data acquisition, standardized preprocessing, glymphatic and susceptibility quantification, and cross-modal analytical strategies. A schematic overview of the framework is shown in Fig. 4.

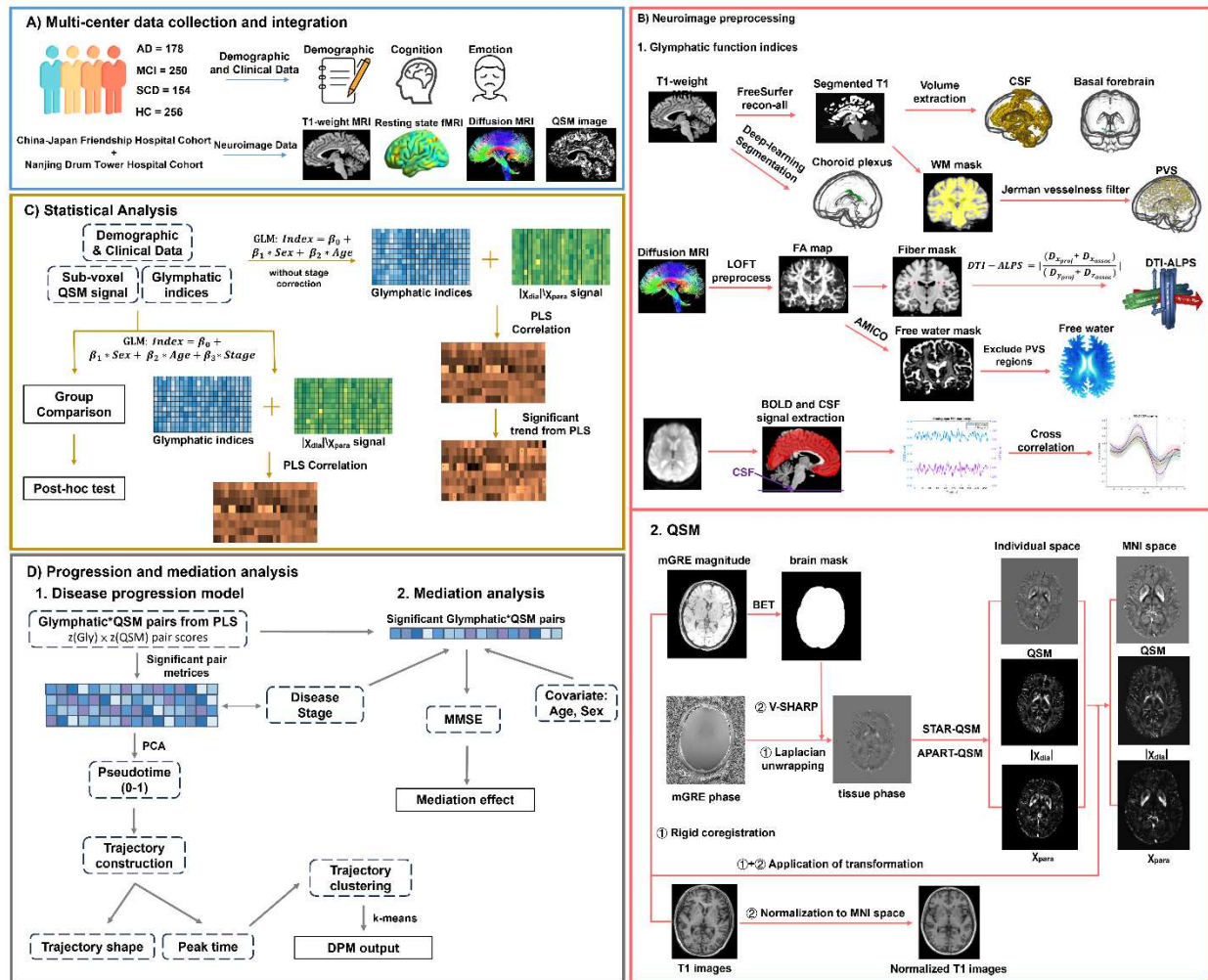


Fig. 4. Overview of study design and multimodal analysis framework. A) Study cohort and multimodal MRI data acquisitions. Participants spanning the HC, SCD, MCI and, AD underwent multimodal MRI acquisition. B)

Complementary glymphatic indices and sub-voxel susceptibility measures, were extracted at both ROI and network levels. MRI preprocessing and quantitative mapping steps are summarized for glymphatic function indices and for susceptibility imaging. B1) Glymphatic indices were derived from diffusion- and CSF-related measures, including, BOLD-CSF coupling, DTI-ALPS, FW, CP, CSF, and BF. B2) Susceptibility features were obtained via QSM reconstruction, enabling joint characterization of glymphatic and susceptibility measures within the same anatomical framework. C) Cross-modal analysis. PLS analyses were used to characterize glymphatic-susceptibility covariance patterns. Additional PLS analysis retaining disease-stage variation was used to identify glymphatic-susceptibility covariance associations with disease stage. D) Stage-related cross-modal features were used to model pseudotemporal trajectories along the AD continuum and to test their mediation effects on the relationship between disease stage and cognitive performance.

4.1 Participants

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of the China-Japan Friendship Hospital (No. 2025-KY-158) and Nanjing Drum Tower Hospital (No. 2023-341-01). Informed consent was obtained from all participants or their relatives who were fully informed about the study.

For the multi-cohort and multimodal MRI study, a total of 838 participants (HC = 256, SCD = 154, MCI = 250, AD = 178) were recruited from the China-Japan Friendship Hospital and Nanjing Drum Tower Hospital. All participants underwent standardized neurological examinations, neuropsychological testing, and a comprehensive MRI protocol. Diagnoses followed the NIA-AA [46], and Petersen criteria [47], and participants were included if they were 50-85 years old, right-handed, and free of major neurological or psychiatric disorders (Fig. 4A). Exclusion criteria comprised significant cerebrovascular lesions, substance misuse, MRI contraindications, or excessive head motion. Some participants underwent abbreviated cognitive screening in the neurology outpatient setting, while some patients with more advanced disease were unable to complete the full neuropsychological battery. In these cases, diagnosis was supported by available clinical assessments together with blood or PET biomarker evidence. Additional demographic information is provided in Supplementary Methods S2.

4.2 Image acquisition

MRI data were acquired on two 3T MR platforms (GE MR750 with an 8-channel head coil at the China-Japan Friendship Hospital; Philips Ingenia CX with a 32-channel head coil at Nanjing Drum Tower Hospital) using site-matched multimodal protocols. 3D T1-weighted FSPGR (1 mm isotropic), 3D multi-echo GRE for QSM (1 mm isotropic), GRE-SS-EPI for resting-state fMRI, and diffusion-weighted spin-echo echo-planar (EPI) sequence for diffusion MRI. Full acquisition parameters for each modality are provided in Supplementary Methods S3.

4.3 MRI data preprocessing

All MRI data underwent standardized preprocessing pipelines to enable reliable extraction of glymphatic-related physiological metrics within a common anatomical framework (details in Supplementary Methods S4.1). Structural T1-weighted images were corrected for bias field inhomogeneity, segmented using FreeSurfer, and registered to MNI152 space via boundary-based

co-registration [48], providing anatomical features of glymphatic function. Diffusion data were denoised, distortion- and motion-corrected, modeled using DTI and bi-tensor free-water estimation, and coregistered to the T1 image [49]. These procedures enabled derivation of perivascular space (ALPS) and free water (FW) indices reflecting perivascular diffusivity and interstitial fluid mobility, which serve as proxies for glymphatic and extracellular transport function. Resting-state fMRI preprocessing included motion correction, nuisance regression, band-pass filtering, and spatial normalization [50], allowing extraction of BOLD-CSF coupling measures that capture neurovascular-driven CSF inflow dynamics.

4.4 Computation of glymphatic measurements and sub-voxel susceptibility

We derived glymphatic-related and susceptibility metrics to characterize brain fluid-transport function and tissue microenvironment properties. All the computational procedures of glymphatic and sub-voxel susceptibility metrics are provided in Supplementary Methods S4.2-S4.4.

BOLD-CSF coupling was derived from resting-state fMRI by quantifying the temporal relationship between ventricular CSF signals and gray matter BOLD fluctuations, with the characteristic negative lag reflecting CSF inflow following neural and vascular activity [11, 12]. Global and temporal BOLD-CSF coupling was included to characterize neurovascular-driven CSF dynamics. DTI-ALPS and FW were used to characterize perivascular transport and interstitial fluid mobility. The DTI-ALPS index quantifies water diffusivity along perivascular directions relative to fiber orientations, providing a proxy for perivascular diffusivity and solute transport efficiency [49, 51]. FW fraction was estimated using the AMICO bi-tensor free-water model from diffusion MRI to quantify extracellular water independent of tissue anisotropy [52]. Structural measures provided complementary information on anatomical compartments and regulatory structures related to brain fluid transport. Volumetric measures of perivascular space (PVS), choroid plexus (CP), ventricular cerebrospinal fluid (CSF) compartment, and basal forebrain (BF) were extracted from structural MRI (Fig. 4B). BF measurements were obtained from the probabilistic JuBrain atlas implemented in the SPM Anatomy Toolbox, using the bilateral Ch4 nucleus and following previously published cholinergic imaging protocols [16]. These measures represent structural correlates of CSF compartments and perivascular pathways that support glymphatic transport [8, 53, 54].

QSM was reconstructed using Laplacian unwrapping, LBV background removal and STAR-QSM inversion, and paramagnetic (χ_{para}) and diamagnetic susceptibility (χ_{dia}) maps were computed through APART-QSM algorithms [37]. These steps enabled separation of χ_{para} and χ_{dia} components reflecting iron-related and diamagnetic macromolecular susceptibility sources. Absolute diamagnetic susceptibility ($|\chi_{\text{dia}}|$) was used to avoid ambiguity in signal interpretation. Higher χ_{para} and $|\chi_{\text{dia}}|$ values indicate greater paramagnetic and diamagnetic tissue content, respectively. All sub-voxel susceptibility maps were subsequently normalized to MNI space via ANTs toolbox [55] to ensure spatial correspondence with glymphatic indices across subjects.

4.5 Region of interests and brain networks selection

To examine spatially specific susceptibility alterations relevant to AD pathology, region of interest (ROI)-level analyses were conducted across cortical and subcortical regions known to be early and frequently affected by A β , tau and iron deposition. Twenty-eight cortical and subcortical ROIs covering AD-vulnerable regions were selected based on prior pathological evidence [2, 28, 38, 56, 57]. All the ROIs were segmented from the T1-QSM hybrid atlas [58]. The A β aggregation related ROIs were superior frontal gyrus (SFG), middle frontal gyrus (MFG), inferior frontal gyrus (IFG), precentral gyrus (PG), occipital gyrus (OG), paracentral lobule (PL), superior parietal lobule (SPL), inferior parietal lobule (IPL), postcentral gyrus (PoG). And, the tau pathology easily affected regions included hippocampus (Hipp), parahippocampal gyrus (PhG), amygdala (Amyg), middle temporal gyrus (MTG), inferior temporal gyrus (ITG), superior temporal gyrus (STG), posterior superior temporal sulcus (Psts), fusiform gyrus (FuG), precuneus (Pcun), cingulate gyrus (CG) and insula (INS). Besides, deep gray matter regions of the caudate nucleus (CN), putamen (Put), globus pallidus (GP), substantia nigra (SN), red nucleus (RN), dentate nucleus (DN), thalamus (Tha) were also selected, as those are the most vulnerable regions for iron alteration in AD.

In addition to these anatomically defined ROIs, QSM measures were examined at the cortical network level to characterize broader cortical susceptibility patterns in relation to glymphatic function, as previous studies have suggested that AD-related pathological and susceptibility alterations exhibit spatial organization across large-scale brain networks [59]. Sub-voxel QSM values in Yeo-7 networks [60] were included for analysis, including frontoparietal (FPN), dorsal attention (DAN), ventral attention (VAN), limbic (LIM), visual (VIS), somatomotor (SOM) and default mode (DMN) networks, as previous studies indicated the aggregation track of AD pathological substances follows the brain networks. Regional χ_{para} and $|\chi_{\text{dia}}|$ values were computed by averaging voxel values within each ROIs and network after masking out CSF and non-brain voxels.

4.6 Statistical Analysis

To characterize glymphatic-susceptibility relationships across the AD continuum, we first examined group-level alterations in individual glymphatic and susceptibility measures. Partial least squares (PLS) analysis was then used to identify multivariate glymphatic-susceptibility relationships [61, 62]. Pairwise correlation analyses were subsequently performed to localize these relationships across specific glymphatic measures and brain regions and to examine their extent across disease stages. Finally, pseudotime analysis was used to characterize how the identified cross-modal features varied along the AD continuum.

4.6.1 Group-Level comparisons of imaging metrics

Group differences in each glymphatic and susceptibility index were tested using ANCOVA, controlling for age, sex, and scanner. Post-hoc pairwise comparisons were FDR-corrected ($q < 0.05$) [63]. Cohen's d was computed to quantify effect sizes. Susceptibility group differences were

545 additionally evaluated after accounting for the corresponding regional gray matter volume (GM)
546 to determine whether tissue atrophy contributed to the observed susceptibility differences.

547 **4.6.2 Multivariate glymphatic-susceptibility covariance analysis**

548 Prior to PLS analysis, glymphatic and susceptibility measures were adjusted for diagnostic stage,
549 age, sex, and scanner. Separate PLS models were constructed for χ_{para} and $|\chi_{\text{dia}}|$. In each model, the
550 predictor matrix consisted of eight glymphatic metrics (gBOLD-CSF coupling, tBOLD-CSF
551 coupling, DTI-ALPS, FW, PVS, CP, CSF, and BF), whereas the response matrix comprised
552 susceptibility features from the 28 ROIs and 7 networks. The adjusted measures were z-score
553 normalized across participants, and PLS decomposed the cross-covariance matrix to identify latent
554 variables (LVs) that maximized shared covariance between glymphatic and susceptibility
555 measures (Fig. 4C).

556 Statistical significance of each latent component was assessed using permutation testing
557 (3,000 permutations), and the stability of feature contributions was evaluated using bootstrap
558 resampling (3,000 resamples). Feature stability was quantified using bootstrap ratios (BR), with
559 glymphatic and susceptibility features showing $|BR| \geq 5$ considered stable contributors to the
560 corresponding LV. Pairwise correlations among the eight glymphatic indices were also examined
561 to assess potential redundancy among predictors, with $|r| > 0.80$ considered indicative of near-
562 collinearity (Supplementary Fig. S1).

563 **4.6.3 Stage-specific glymphatic-susceptibility correlations**

564 For the stage-specific analysis, additional PLS models were constructed without adjustment for
565 diagnostic stage to retain cross-modal variation associated with disease stage. Stable glymphatic
566 and susceptibility features identified from significant LVs were then used for stage-specific
567 correlation analyses. Significant glymphatic and susceptibility features from the PLS analysis were
568 standardized and multiplied to generate cross-modal features [$z(\text{glymphatic}) \times z(\text{susceptibility})$].
569 All pairwise combinations of stable glymphatic and susceptibility features identified from
570 significant PLS LVs were examined separately within the HC, SCD, MCI, and AD groups.
571 Correlations were adjusted for age, sex, and scanner, and FDR correction ($\text{FDR } q < 0.001$ and η^2
572 > 0.06) was applied for multiple comparisons. Significant correlations within each diagnostic
573 group were used to characterize the stage expression and extent of the cross-modal relationships
574 across the AD continuum (Fig. 4C).

575 **4.6.4 Pseudotime-based disease progression modelling (DPM)**

576 To characterize variation in glymphatic-susceptibility relationships along the AD continuum,
577 pseudotime analysis was performed [43, 44]. Significant glymphatic–susceptibility pairs from the
578 stage-specific correlation analysis were retained across the χ_{para} and $|\chi_{\text{dia}}|$ ROI- and network-level
579 models. The resulting features were combined into a subject-by-feature matrix with z-score
580 normalization.

Subject-level pseudotime was estimated as the first principal component of the standardized subject-by-feature matrix composed of pair-derived cross-modal features. The resulting pseudotime scores were oriented toward increasing disease severity and rescaled to the [0,1] interval. Feature trajectories were estimated by ordering subjects along pseudotime, interpolating and smoothing feature values across the pseudotime axis. For each cross-modal feature, the relative timing of alteration was characterized based on peak pseudotime and overall trajectory shape. K-means clustering using correlation distance was subsequently applied to group features into distinct temporal classes, enabling characterization of heterogeneous progression patterns (Fig. 4D). Clusters were characterized according to their relative temporal positions along the pseudotime axis, enabling identification of early-, intermediate-, and late-altering cross-modal patterns. To identify significant pseudotemporal cross-modal patterns, features were required to have a consistent cluster pattern and pronounced variation along pseudotime (silhouette score > 0; trajectory range > cluster median; Fig. 4D).

4.6.5 Mediation analysis of cross-modal features and cognitive performance

Mediation analyses were employed to test whether significant cross-modal features mediated the relationship between disease stage and cognitive performance (MMSE). For each cross-modal feature, disease stage was specified as the predictor, the pair-derived cross-modal score as the mediator, and MMSE as the outcome. Age, sex, and scanner were included as covariates. Indirect effects were estimated using bootstrap resampling.

5. Data availability

The data are not publicly available because the study is ongoing. Public sharing is restricted by participant consent, institutional ethics approval, and institutional data-governance policies. De-identified data supporting the findings may be requested from the corresponding author, Guolin Ma (maguolin1007@qq.com), for scientifically sound research purposes. An initial response will normally be provided within 30 days.

6. Code availability

The code used for data processing and statistical analyses is available from the corresponding author upon reasonable request. Open-source software was used for all analyses.

7. References:

1. Bloom, G.S., *Amyloid- β and tau: the trigger and bullet in Alzheimer disease pathogenesis*. JAMA neurology, 2014. **71**(4): p. 505-508.

2. Jagust, W., *Imaging the evolution and pathophysiology of Alzheimer disease*. Nature Reviews Neuroscience, 2018. **19**(11): p. 687-700.
3. Weiner, M.W., et al., *The Alzheimer's Disease Neuroimaging Initiative: a review of papers published since its inception*. Alzheimer's & Dementia, 2013. **9**(5): p. e111-e194.
4. Apátiga-Pérez, R., et al., *Neurovascular dysfunction and vascular amyloid accumulation as early events in Alzheimer's disease*. Metabolic brain disease, 2022. **37**(1): p. 39-50.
5. Jessen, N.A., et al., *The glymphatic system: a beginner's guide*. Neurochemical research, 2015. **40**: p. 2583-2599.
6. Tarasoff-Conway, J.M., et al., *Clearance systems in the brain—implications for Alzheimer disease*. Nature reviews neurology, 2015. **11**(8): p. 457-470.
7. Choi, J.D., et al., *Choroid plexus volume and permeability at brain MRI within the Alzheimer disease clinical spectrum*. Radiology, 2022. **304**(3): p. 635-645.
8. Lin, W., et al., *Multimodal MRI reveals impaired glymphatic function with choroid plexus enlargement and cerebrospinal fluid expansion in alzheimer's disease*. Scientific Reports, 2025. **15**(1): p. 30409.
9. Costa, T., et al., *Evaluating the robustness of DTI-ALPS in clinical context: a meta-analytic parallel on Alzheimer's and Parkinson's diseases*. Scientific Reports, 2024. **14**(1): p. 26381.
10. Kiviniemi, V., et al., *Ultra-fast magnetic resonance encephalography of physiological brain activity—glymphatic pulsation mechanisms?* Journal of Cerebral Blood Flow & Metabolism, 2016. **36**(6): p. 1033-1045.
11. Fultz, N.E., et al., *Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep*. Science, 2019. **366**(6465): p. 628-631.
12. Han, F., et al., *Reduced coupling between cerebrospinal fluid flow and global brain activity is linked to Alzheimer disease–related pathology*. PLoS biology, 2021. **19**(6): p. e3001233.
13. Steward, C.E., et al., *Assessment of the DTI-ALPS parameter along the perivascular space in older adults at risk of dementia*. Journal of Neuroimaging, 2021. **31**(3): p. 569-578.
14. Yu, P., L. Shen, and L. Tang, *Multimodal DTI-ALPS and Hippocampal Microstructural Signatures Unveil Stage-Specific Pathways in Alzheimer's Disease Progression*. Frontiers in Aging Neuroscience, 2025. **17**: p. 1609793.
15. Zhang, Y., et al., *DTI-ALPS index-assessed glymphatic dysfunction mediates Alzheimer's cognitive decline via amyloid- β -dependent pathways: multimodal PET/MRI study*. European Journal of Nuclear Medicine and Molecular Imaging, 2025: p. 1-13.
16. Chuang, K.-H., et al., *Cholinergic basal forebrain neurons regulate vascular dynamics and cerebrospinal fluid flux*. Nature Communications, 2025. **16**(1): p. 5343.
17. Iliff, J.J., et al., *A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β* . Science translational medicine, 2012. **4**(147): p. 147ra111-147ra111.
18. Murdock, M.H., et al., *Multisensory gamma stimulation promotes glymphatic clearance of amyloid*. Nature, 2024. **627**(8002): p. 149-156.
19. Xie, L., et al., *Sleep drives metabolite clearance from the adult brain*. science, 2013. **342**(6156): p. 373-377.
20. Conrad, M., et al., *Regulated necrosis: disease relevance and therapeutic opportunities*. Nature reviews Drug discovery, 2016. **15**(5): p. 348-366.

21. Everett, J., et al., *Evidence of redox-active iron formation following aggregation of ferrihydrite and the Alzheimer's disease peptide β -amyloid*. Inorganic Chemistry, 2014. **53**(6): p. 2803-2809.
22. Tao, Y., et al., *Perturbed iron distribution in Alzheimer's disease serum, cerebrospinal fluid, and selected brain regions: a systematic review and meta-analysis*. Journal of Alzheimer's Disease, 2014. **42**(2): p. 679-690.
23. Zecca, L., et al., *Iron, brain ageing and neurodegenerative disorders*. Nature Reviews Neuroscience, 2004. **5**(11): p. 863-873.
24. Becerril-Ortega, J., et al., *Iron overload accelerates neuronal amyloid- β production and cognitive impairment in transgenic mice model of Alzheimer's disease*. Neurobiology of aging, 2014. **35**(10): p. 2288-2301.
25. Schweser, F., et al., *Quantitative imaging of intrinsic magnetic tissue properties using MRI signal phase: an approach to in vivo brain iron metabolism?* Neuroimage, 2011. **54**(4): p. 2789-2807.
26. Liu, C., et al., *Quantitative susceptibility mapping: contrast mechanisms and clinical applications*. Tomography, 2015. **1**(1): p. 3.
27. Lee, J., S. Ji, and S.-H. Oh, *So you want to image myelin using MRI: magnetic susceptibility source separation for myelin imaging*. Magnetic Resonance in Medical Sciences, 2024. **23**(3): p. 291-306.
28. Ahmed, M., et al., *The diamagnetic component map from quantitative susceptibility mapping (QSM) source separation reveals pathological alteration in Alzheimer's disease-driven neurodegeneration*. Neuroimage, 2023. **280**: p. 120357.
29. Uchida, Y., et al., *APOE ϵ 4 dose associates with increased brain iron and β -amyloid via blood-brain barrier dysfunction*. Journal of Neurology, Neurosurgery & Psychiatry, 2022. **93**(7): p. 772-778.
30. Uchida, Y., et al., *Contributions of blood-brain barrier imaging to neurovascular unit pathophysiology of Alzheimer's disease and related dementias*. Frontiers in Aging Neuroscience, 2023. **15**: p. 1111448.
31. Wei, J., et al., *Glymphatic dysfunction contributes to thalamic iron retention and secondary thalamic injury after stroke: evidence from primates and rodents*. Journal of Neuroinflammation, 2026.
32. Zhou, W., et al., *Dysfunction of the glymphatic system might be related to iron deposition in the normal aging brain*. Frontiers in aging neuroscience, 2020. **12**: p. 559603.
33. Yao, J., et al., *Distinct regional vulnerability to A β and iron accumulation in post mortem AD brains*. Alzheimer's & Dementia, 2024. **20**(10): p. 6984-6997.
34. Ayton, S., et al., *Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology*. Molecular psychiatry, 2020. **25**(11): p. 2932-2941.
35. van der Weijden, C.W., et al., *Quantitative myelin imaging with MRI and PET: an overview of techniques and their validation status*. Brain, 2023. **146**(4): p. 1243-1266.
36. Liu, P., et al., *Layer-specific changes in sensory cortex across the lifespan in mice and humans*. Nature neuroscience, 2025. **28**(9): p. 1978-1989.
37. Li, Z., et al., *APART-QSM: An improved sub-voxel quantitative susceptibility mapping for susceptibility source separation using an iterative data fitting method*. Neuroimage, 2023. **274**: p. 120148.

38. Buckner, R.L., et al., *Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory*. Journal of neuroscience, 2005. **25**(34): p. 7709-7717.
39. Duyn, J.H. and J. Schenck, *Contributions to magnetic susceptibility of brain tissue*. NMR in Biomedicine, 2017. **30**(4): p. e3546.
40. Hablitz, L.M., et al., *Increased glymphatic influx is correlated with high EEG delta power and low heart rate in mice under anesthesia*. Science advances, 2019. **5**(2): p. eaav5447.
41. Taoka, T. and S. Naganawa, *Glymphatic imaging using MRI*. Journal of Magnetic Resonance Imaging, 2020. **51**(1): p. 11-24.
42. Ota, M., et al., *Relationships between the deposition of amyloid- β and tau protein and glymphatic system activity in Alzheimer's disease: diffusion tensor image study*. Journal of Alzheimer's Disease, 2022. **90**(1): p. 295-303.
43. Donohue, M.C., et al., *Estimating long-term multivariate progression from short-term data*. Alzheimer's & Dementia, 2014. **10**: p. S400-S410.
44. Trapnell, C., et al., *The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells*. Nature biotechnology, 2014. **32**(4): p. 381-386.
45. Bouter, Y., et al., *18F-FDG-PET and Multimodal Biomarker Integration: a Powerful Tool for Alzheimer's Disease Diagnosis*. Nuclear Medicine and Molecular Imaging, 2025: p. 1-19.
46. Jack Jr, C.R., et al., *NIA-AA research framework: toward a biological definition of Alzheimer's disease*. Alzheimer's & dementia, 2018. **14**(4): p. 535-562.
47. Petersen, R.C., et al., *Mild cognitive impairment: clinical characterization and outcome*. Archives of neurology, 1999. **56**(3): p. 303-308.
48. Fischl, B., *FreeSurfer*. Neuroimage, 2012. **62**(2): p. 774-781.
49. Liu, X., et al., *Cross-vendor test-retest validation of diffusion tensor image analysis along the perivascular space (DTI-ALPS) for evaluating glymphatic system function*. Aging and disease, 2024. **15**(4): p. 1885.
50. Gu, Y., et al., *Transient arousal modulations contribute to resting-state functional connectivity changes associated with head motion parameters*. Cerebral Cortex, 2020. **30**(10): p. 5242-5256.
51. Taoka, T., et al., *Diffusion tensor image analysis ALong the perivascular space (DTI-ALPS): revisiting the meaning and significance of the method*. Magnetic Resonance in Medical Sciences, 2024: p. rev. 2023-0175.
52. Daducci, A., et al., *Accelerated microstructure imaging via convex optimization (AMICO) from diffusion MRI data*. Neuroimage, 2015. **105**: p. 32-44.
53. Eisma, J.J., et al., *Deep learning segmentation of the choroid plexus from structural magnetic resonance imaging (MRI): validation and normative ranges across the adult lifespan*. Fluids and Barriers of the CNS, 2024. **21**(1): p. 21.
54. Lin, S., et al., *Association of MRI indexes of the perivascular space network and cognitive impairment in patients with obstructive sleep apnea*. Radiology, 2024. **311**(3): p. e232274.
55. Klein, A., et al., *Evaluation of volume-based and surface-based brain image registration methods*. Neuroimage, 2010. **51**(1): p. 214-220.
56. Kim, H.-G., et al., *Quantitative susceptibility mapping to evaluate the early stage of Alzheimer's disease*. NeuroImage: Clinical, 2017. **16**: p. 429-438.
57. Hampel, H., et al., *The amyloid- β pathway in Alzheimer's disease*. Molecular psychiatry, 2021. **26**(10): p. 5481-5503.

58. Zhang, Y., et al., *Longitudinal atlas for normative human brain development and aging over the lifespan using quantitative susceptibility mapping*. Neuroimage, 2018. **171**: p. 176-189.
59. Chen, H., et al., *Associations of quantitative susceptibility mapping with cortical atrophy and brain connectome in Alzheimer's disease: A multi-parametric study*. NeuroImage, 2024. **290**: p. 120555.
60. Yeo, B.T., et al., *The organization of the human cerebral cortex estimated by intrinsic functional connectivity*. Journal of neurophysiology, 2011.
61. Krishnan, A., et al., *Partial Least Squares (PLS) methods for neuroimaging: a tutorial and review*. Neuroimage, 2011. **56**(2): p. 455-475.
62. Cha, J., *Partial least squares*. Adv. Methods Mark. Res, 1994. **407**: p. 52-78.
63. Benjamini, Y. and Y. Hochberg, *Controlling the false discovery rate: a practical and powerful approach to multiple testing*. Journal of the Royal statistical society: series B (Methodological), 1995. **57**(1): p. 289-300.

8. Author contribution

CT.L.: conceptualization, methodology, validation, investigation, formal analysis, writing—original draft, writing—review and editing. AC.Y.: conceptualization, methodology, validation, investigation, formal analysis, writing—original draft, writing—review and editing. RS.W.: investigation, formal analysis. Y.S.: resources, data curation, validation. JX.L.: methodology, validation. MX.X.: methodology, validation, formal analysis. Y.G.: resources, data curation, validation. HW.Y.: resources, data curation, validation. YL.W.: resources, data curation, validation. N.S.: writing—review and editing. B. Z.: writing—review and editing. GL.M.: conceptualization resources, funding acquisition, project administration, writing—review and editing. Z.Y.: conceptualization resources, funding acquisition, project administration, writing—review and editing.

9. Competing interests

The authors declare no conflicts of interest

10. Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Committee of the China-Japan Friendship Hospital (No. 2025-KY-158) and Nanjing Drum Tower Hospital (No. 2023-341-01). All the participants and/or their relatives were informed about this study and provided their written informed consent.

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