Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction

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Vascular contributions to cognitive impairment are increasingly recognized as shown by neuropathological, neuroimaging, and cerebrospinal fluid biomarker studies. Moreover, small vessel disease of the brain has been estimated to contribute to approximately 50% of all dementias worldwide, including those caused by Alzheimer’s disease (AD).

Vascular changes in AD have been typically attributed to the vasoactive and/or vasculo-toxic effects of amyloid-β (Aβ) and more recently tau. Animal studies suggest that Aβ and tau lead to blood vessel abnormalities and blood–brain barrier (BBB) breakdown. Although neurovascular dysfunction and BBB breakdown develop early in AD, how they relate to changes in the AD classical biomarkers Aβ and tau, which also develop before dementia, remains unknown. To address this question, we studied brain capillary damage using a novel cerebrospinal fluid biomarker of BBB-associated capillary mural cell pericyte, soluble platelet-derived growth factor receptor-β (sPDGFRβ), and regional BBB permeability using dynamic contrast-enhanced magnetic resonance imaging (DCE) magnetic resonance imaging (MRI) measures of blood–brain barrier (BBB) dysfunction. Consistent with findings that a disintegrin and metalloproteinase 10 (ADAM10) sheds sPDGFRβ in fibroblasts, we found that ADAM10 mediates sPDGFRβ shedding in human pericytes but not SMCs (Extended Data Fig. 1), supporting the hypothesis that sPDGFRβ is a biomarker of brain capillary pericytes.

We found increased CSF sPDGFRβ with more advanced CDR (CDR 1 > 0.5 > 0) (Fig. 1a), suggesting progressive damage of pericytes with cognitive dysfunction. There were no significant differences in CSF Aβ42 or pTau levels between CDR 0.5 and CDR 0 individuals, although we saw reduced CSF Aβ42 in CDR 1 relative to CDR 0.5 participants (Fig. 1b,c; for site-specific analysis, see Extended Data Fig. 2a,b). sPDGFRβ was increased in participants with CDR 0.5 relative to CDR 0 regardless of CSF Aβ42 (Fig. 1d) or pTau (Fig. 1e) status, that is, irrespective of Aβ+ or Aβ−, or pTau+ or pTau−, as confirmed by site-specific analyses (Extended Data Fig. 2c,d). Higher CSF sPDGFRβ remained a significant predictor of cognitive impairment after statistically controlling for CSF Aβ42 and pTau, as shown by the estimated marginal means from the analysis of covariance (ANCOVA) models (Fig. 1f), indicating medium-to-large incremental effect sizes with $r^2_{partial}$ range = 0.10–0.12, which has been confirmed by logistic regression.

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models (Supplementary Table 3a–c). There was a significant positive correlation between CSF sPDGFβ with classical biomarkers of BBB breakdown, including the CSF/plasma albumin ratio and CSF fibrinogen (Extended Data Fig. 2c,f). Among the subset of 35 participants who underwent Pittsburgh compound B (PiB)-positron emission tomography (PET), those with CDR 0.5 exhibited increased CSF sPDGFβ relative to those with CDR 0, after statistically controlling for amyloid levels (Extended Data Fig. 2g), consistent with CSF Aβ42 findings (Fig. 1d). Additionally, we found no differences in CSF Aβ and tau oligomer levels between the CDR 0 and CDR 0.5 groups (Extended Data Fig. 2h,i). CSF sPDGFβ remained significantly elevated in CDR analysis after statistically controlling for CSF tau oligomers in ANCOVA models (Extended Data Fig. 2j,k). Regional Ktrans values controlled for CSF Aβ and pTau levels in CDR 0 (n = 44) and CDR 0.5 (n = 23) individuals. Estimated marginal means ± s.e.m. from ANCOVA models. The box and whisker plot lines indicate the median values, the boxes indicate the interquartile range (IQR) and the whiskers indicate the minimum and maximum values. Significance tests after FDR correction from ANCOVA with post-hoc Bonferroni comparisons.

pericytes, which critically maintain the BBB integrity, develops early in older adults with cognitive dysfunction, is independent of Aβ and tau biomarker changes, is not influenced by VRFs, and is not associated with the glial and/or inflammatory response, or detectable neuronal degeneration.

The DCE-MRI analysis of regional BBB permeability in a subset of 73 participants with CDR 0.5 compared to those with CDR 0, indicated increased BBB permeability to gadolinium-based contrast agent in the hippocampus (HC) and its CA1, CA3, and dentate gyrus subfields, and parahippocampal gyrus (PHG), but not in other studied brain regions including the frontal and temporal cortex, subcortical white matter, corpus callosum, and internal capsule, and deep gray matter regions including the thalamus and striatum (Extended Data Fig. 5a,b). These findings are consistent with a recent report demonstrating that BBB breakdown during normal aging and mild cognitive impairment starts in the HC. Surprisingly, we also found that individuals with a CDR 0.5 compared to those who were cognitively normal (CDR 0) exhibited BBB breakdown in the HC, PHG, and HC subfields, regardless of CSF Aβ42 (Fig. 1g,h) and pTau (Fig. 1i,j) status. Increased regional BBB permeability in the HC, PHG, and HC subfields remained a significant predictor of cognitive impairment after statistically controlling for CSF Aβ42 and pTau, as shown by the estimated marginal means from ANCOVA models (Fig. 1k) indicating medium-to-large incremental effect sizes ($\eta^2_{partial} range = 0.09–0.28$), also confirmed by logistic regression models (Supplementary Table 3d–h).
Nevertheless, the fact that brain capillary mural cell damage and tau status does not affect BBB integrity in other studied brain regions (Extended Data Fig. 5e,d). Similar to the sPDGFRβ findings, VRF burden did not influence BBB permeability changes in the HC and PHG in individuals with CDR 0.5 compared to CDR 0, and had no effect on BBB integrity in other studied brain regions (Extended Data Fig. 5e,f). Consistent with previous findings, in the present cohort we also observed a significant positive correlation between increases in CSF sPDGFRβ and DCE-MRI measures of BBB permeability in the HC and PHG in all studied participants (Extended Data Fig. 5g,h), which was not the case for other studied brain regions, as illustrated in this study for the white matter regions (Extended Data Fig. 5i,j).

Because the present study sample excluded participants with vascular dementia and vascular cognitive impairment and substantial cerebrovascular pathology, it is probably not surprising that BBB dysfunction in the present analysis was independent of traditional systemic VRFs. However, the possibility of interactive or synergistic effects of traditional VRFs and BBB dysfunction in populations with more severe vascular lesions, vascular dementia, and vascular cognitive impairment is not ruled out by the present findings. Nevertheless, the fact that brain capillary mural cell damage and BBB breakdown is independent of traditional VRFs, as we show, is critical information that underscores the heterogeneity of vascular pathologies in the aging brain.

To address whether changes in CSF sPDGFRβ and DCE-MRI BBB permeability measures depend on HC volume, we conducted ANCOVA analyses and hierarchical logistic regression correcting for FreeSurfer-derived HC and/or PHG volumes (Extended Data Fig. 5a). In participants with CDR 0.5 versus CDR 0, we found no significant changes in HC volume, but a significant decrease in PHG volume (Fig. 2b). HC or PHG volumes did not statistically differ between participants that were CSF sPDGFRβ versus Aβ (Fig. 2c) or pTau versus pTau (Fig. 2d) in either the CDR 0 or CDR 0.5 groups. Importantly, CSF sPDGFRβ increases remained significant after controlling for HC and PHG volumes (estimated marginal means ± s.e.m. from ANCOVA models) (Fig. 2e), and remained increased when stratifying by CSF Aβ and pTau status (Fig. 2f). Similarly, the HC and PHG BBB permeability increases remained significant after controlling for HC and PHG volumes, respectively (Fig. 2g), and when stratifying by Aβ and pTau status (Fig. 2h). All findings exhibited medium-to-large incremental effect sizes after controlling for HC and PHG volume ($r_{\text{partial}}$ range = 0.09–0.31) and were corroborated by logistic regression models (Supplementary Table 4a–c). Collectively,
these data suggest that BBB impairment that is represented by CSF sPDGFβ and DCE-MRI measures is not only independent of CSF AD biomarkers, but is also not correlated to HC volume.

To determine whether our findings hold when cognitive dysfunction was evaluated by neuropsychological performance, we analyzed CSF biomarkers and BBB integrity using normalized scores from...
Fig. 4 | Early brain capillary damage and BBB breakdown in human HC and PHG in individuals with an increased cognitive domain impairment is independent of HC and PHG volume. a–c, Bilateral HC and PHG volumes in individuals with 0 (n = 44) and 1+ (n = 24) impaired cognitive domains (a), Aβ− (n = 25) or Aβ+ (n = 18) with 0 impaired domains and Aβ− (n = 9) or Aβ+ (n = 7) with 1+ impaired domains (b), and pTau− (n = 32) or p Tau+ (n = 11) with 0 impaired domains and pTau− (n = 13) or p Tau+ (n = 7) with 1+ impaired domains (c). d–f, CSF sPDGFRβ controlled for HC and PHG volume in individuals with 0 (n = 38) and 1+ impaired domains (n = 21) (d), and Aβ− (n = 30) or Aβ+ (n = 12) 0 impaired domains and Aβ− (n = 15) or Aβ+ (n = 7) 1+ impaired domains (e), and p Tau− (n = 30) or p Tau+ (n = 12) 0 impaired domains and p Tau− (n = 13) or p Tau+ (n = 7) 1+ impaired domains (f). Estimated marginal means ± s.e.m. from ANCOVA models. g–i, BBB Ktrans values in the HC and PHG controlled for respective HC or PHG volume in individuals with 0 (n = 44) and 1+ impaired domains (n = 24) (g), and in the HC and PHG controlled for respective HC or PHG volume in participants who are Aβ− (n = 24) or Aβ+ (n = 18) 0 impaired domains and Aβ− (n = 12) or Aβ+ (n = 10) 1+ impaired domains (h), and p Tau− (n = 30) or p Tau+ (n = 12) 0 impaired domains and p Tau− (n = 13) or p Tau+ (n = 7) 1+ impaired domains (i). Estimated marginal means ± s.e.m. from ANCOVA models. The box and whisker plots indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. Significance tests after FDR correction from ANCOVAs with post-hoc Bonferroni comparisons.

ten neuropsychological tests used to evaluate impairment in memory, attention/executive function and language, and global cognition, as described in the online Methods. This analysis indicated elevated CSF sPDGFRβ in participants with one impaired cognitive domain relative to those with no impaired domains (Fig. 3a; see Extended Data Fig. 6a,b for site-specific analyses). However, there was no difference in CSF Aβ42 between participants with one impaired domain and those with no impaired domains (Fig. 3b), although participants with one impaired domain showed increased CSF p Tau relative to those with no impaired domains (Fig. 3c).

Stratification of participants into those with and without classic AD biomarker abnormalities revealed increased CSF sPDGFRβ in participants with one or more impaired domains regardless of CSF Aβ42 (Fig. 3d) or p Tau (Fig. 3e) status (see Extended Data Fig. 6c–d for site-specific analyses), or VRFs burden, as shown in the entire sample and confirmed by site-specific analysis (Extended Data Fig. 6e–g). Higher CSF sPDGFRβ levels remained a significant predictor of cognitive impairment after statistically controlling for CSF Aβ42 and p Tau, as shown by the estimated marginal means from the ANCOVA models (Fig. 3f) indicating medium-to-large incremental effect sizes (η2 partial range = 0.07–0.14), which has been confirmed by logistic regression models at both sites (Supplementary Table 5a–c).

Similar to the CDR analysis, in the subset of participants who underwent PiB-PET scans, participants with domain impairment exhibited increased CSF sPDGFRβ relative to those without impairment, after statistically controlling for amyloid levels (Extended Data Fig. 7a), thus corroborating the CSF Aβ42 data (Fig. 3d). There was no difference in CSF Aβ and tau oligomers between participants with impairment in one or more cognitive domains and those without cognitive impairment (Extended Data Fig. 7b,c). CSF sPDGFRβ remained significantly increased in domain analysis after statistically controlling for CSF tau oligomers in the ANCOVA models (Extended Data Fig. 7d).

There were no differences in CSF markers of glial and/or inflammatory response, or neuronal degeneration, between impaired and unimpaired participants on neuropsychological exams, as illustrated by a few examples (Extended Data Fig. 8a; also confirmed by site-specific analysis in Extended Data Fig. 8b,c).
Among participants undergoing DCE-MRI scans, those with domain impairment relative to those without impairment exhibited BBB breakdown in the HC, PHG, and HC subfields, but not in other studied brain regions (Extended Data Fig. 9a,b) regardless of CSF Aβ$_42$ (Fig. 3g–h; Extended Data Fig. 9c), pTau status (Fig. 3i–j; Extended Data Fig. 9d), or VRF status (Extended Data Fig. 9e,f). Increased regional BBB permeability in HC, PHG, and HC subfields remained a significant predictor of cognitive impairment after statistically controlling for CSF Aβ$_42$ and pTau, as shown by the estimated marginal means from the ANCOVA models (Fig. 1k), indicating medium-to-large incremental effect sizes ($\eta^2_{\text{partial}}$ range = 0.07–0.18), also confirmed by logistic regression analysis (Supplementary Table 5d–h).

An increase in DCE-MRI BBB permeability in several medial temporal lobe structures that subserve episodic memory (for example, HC, PHG, and the CA1, CA3, and dentate gyrus HC subfields) was associated with worse CDR scores (CDR 0 versus 0.5) and with impairment in multiple cognitive domains (impairment in 0 versus 1+ domains) (Fig. 1g–k; Fig. 3g–k). Although this provides a perfect anatomical substrate for episodic memory impairment, it is less clear whether BBB pathology in the HC and medial temporal lobe can contribute to changes seen in other domains in participants with CDR 0.5 or with impairment in multiple domains, which involves areas of the brain outside the medial temporal lobe that we found were not affected by BBB breakdown in the present cohort (Extended Data Fig. 5a,b and 9a,b). However, numerous studies have linked HC structure and function to each of the cognitive domains and subdomains investigated in the present study. For example, experimental studies in animals and observational human studies have found that attention, working memory, and executive function can become dysfunctional as a result of HC-prefrontal pathway disruption26–28. HC functional activation has been found to underpin normal performance on semantic fluency tasks39; neuroimaging-based markers of HC structure and function correlate with performance on semantic fluency and confrontation naming tasks in both normal and pathological human aging29. Thus, BBB breakdown within the HC and medial temporal regions may disrupt the ability of these structures and their connecting pathways to support an array of cognitive functions. Additionally, we noted increased BBB permeability in the caudate nucleus (Extended Data Fig. 5a,b and 9a,b), a structure known to support the frontal-subcortical processes involved in attention/executive functions and verbal fluency29,30. Although less salient than the HC and PHG findings, it is possible that BBB breakdown within the caudate nucleus may contribute to the observed deficits in domains beyond memory.

As with CDR analysis, there were no significant changes in HC volume, but a significant decrease in PHG volume (Fig. 1f), indicating medium-to-large incremental effect sizes ($\eta^2_{\text{partial}}$ range = 0.19–0.25) and were corroborated by logistic regression models (Supplementary Table 6a–c). Finally, we asked the following question. Did CSF sPDGFRβ and DCE-MRI BBB increases correlate with age? Neither CSF sPDGFRβ (Extended Data Fig. 10a,b) nor regional BBB permeability HC and PHG values (Extended Data Fig. 10c–f) were correlated with age in either the CDR 0 or CDR 0.5 groups. Since all CDR and domain impairment group differences in CSF sPDGFRβ and in HC and PHG BBB permeability values were significant after correcting for age (Fig. 1; Fig. 3), these data indicate that CSF sPDGFRβ and HC and PHG BBB measures reflect cognitive impairment independent of normal aging; therefore, they may be good biomarkers of early cognitive dysfunction.

In summary, we show that older adults with early cognitive dysfunction develop brain capillary damage associated with mural cell pericyte injury and BBB breakdown in the HC irrespective of Aβ and/or tau changes, suggesting that BBB breakdown is an independent, early biomarker of cognitive impairment unrelated to Aβ and tau. The independence of the BBB breakdown pathway from the Aβ/tau pathway in predicting cognitive impairment is further supported by logistic regression models indicating that BBB breakdown is not mediating the relationship between AD biomarkers and cognitive impairment (Supplementary Tables 7–10). Biomarker-based diagnostic approaches, including the recent research recommendations for AD1–3, mention vascular biomarkers, but suggest that CSF Aβ$_42$ and pTau, and amyloid PET and tau PET, are the key biomarkers defining AD pathology, although they may not be causal to the disease process47–49. Our present findings support that neurovascular dysfunction may represent a previously underestimated factor contributing to cognitive and functional decline, independent of the classic pathophysiological hallmarks of AD. Moreover, our findings point to the brain vasculature as an important new biomarker of cognitive dysfunction in both individuals without and with Aβ or pTau positivity, the latter indicating individuals in the Alzheimer's continuum49.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41591-018-0297-y.

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Author contributions

D.A.N., M.D.S., A.M., P.S., and B.V.Z. designed the research study and analyzed and interpreted the data. M.D.S., A.M., P.S., M.P., and D.P.B. performed the experiments and analyzed the data. L.M.D. and A.R.N. prepared and submitted the study to the IRB. M.G.H., T.L.S.B., A.M.F., J.M.R., L.S.S., J.C.M., H.C.C., M.L., and A.W.T. recruited the participants and performed and provided the imaging scans. A.P.S., M.G.H., T.L.S.B., A.M.F., J.M.R., L.S.S., J.C.M., H.C.C., M.L., and A.W.T. provided critical reading of the manuscript. D.A.N., M.D.S., and A.M. contributed to manuscript writing and R.V.Z. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Study participants. Participants were recruited from two sites, including the University of Southern California (USC), Los Angeles, and Washington University, St. Louis. At the USC site, participants were recruited through the USC Alzheimer’s Disease Research Center (ADRC); combined USC, Huntington Medical Research Institutes in Pasadena. At the Washington University site, participants were recruited through the Washington University Knight ADRC. The study and procedures were approved by the Institutional Review Board (IRB) of the USC ADRC and Washington University Knight ADRC indicating compliance with all ethical regulations; informed consent was obtained from all participants before study enrollment. Participants from both sites were included in CSF biomarker studies. All participants underwent neurological and neuropsychological evaluations performed using the Uniform Data Set (UDS), and additional neuropsychological tests, as described in the following sections. Participants from the USC ADRC were included in DCE-MRI studies for assessment of BBB permeability if they had no contraindications for contrast injection or MR imaging. We included 161 participants for the CSF biomarker studies (74 from USC/ Huntington Medical Research Institutes and 87 from Washington University). A group of 35 participants from the Washington University Knight ADRC underwent PiB-PET imaging for amyloid deposition. A group of 73 participants recruited from the USC ADRC underwent DCE-MRI. All biomarker assays and quantitative MRI scans were conducted by investigators blinded to the clinical status of the participant.

Inclusion and exclusion criteria. Included participants (≥245 years of age) with neuropathologically confirmed cognition, and/or early cognitive dysfunction and/or AD, or prior history of any neurological or psychiatric conditions that might better account for any observed cognitive impairment, including organ failure, brain tumors, epilepsy, hydrocephalus, schizophrenia, and major depression. Participants were stratified based on CSF analysis as either Aβ+ (Aβ42, <190 pg/ml) or Aβ− (Aβ42, >190 pg/ml), or pTau441, positive (pTau441, >78 pg/ml) or pTau441, negative (pTau441, <78 pg/ml). Participants were excluded if they were diagnosed with vascular cognitive impairment or vascular dementia. These clinical diagnoses were conducted by neurologists based on the criteria of whether the patient (1) had a known vascular brain injury and (2) the clinician judged that the vascular brain injury played a role in their cognitive impairment, and/or pattern and course of symptoms. In addition to clinical diagnosis, presence of vascular lesions was confirmed by moderate-to-severe white matter changes and lacunar infarcts by fluid-attenuated inversion recovery MRI and/or subcortical microbleeds by T2*-weighted MRI. Participants were also excluded if they were diagnosed with Parkinson's disease, Lewy body dementia, or frontotemporal dementia. History of a single stroke or transient ischemic attack was not an exclusion unless it was related to symptomatic onset of cognitive impairment. Participants also did not have current contraindications to MRI and were not currently using medications that might better account for any observed cognitive impairment.

CDR. CDR assessments followed the standardized UDS procedures. Participants underwent clinical interview, including health history, and a physical examination. Knowledgeable informants were also interviewed. Given the lack of scientific consensus regarding the categorization of older adults along the aging to MCI to AD dementia spectrum and the time course and sequence of biomarker changes, we determined consensus regarding the categorization of older adults along the aging to MCI to AD dementia spectrum and the time course and sequence of biomarker changes.

Neuropsychological evaluation and domain impairment. Neuropsychological performance was used to identify domain impairment. All participants underwent neuropsychological testing using the UDS battery (version 2.0 or 3.0) or supplemental neuropsychological tests at each site. Test impairment for the UDS tests was determined using age-, sex-, and education-corrected scores from the National Alzheimer’s Coordinating Center (www.alz.washington.edu). Normalized scores from the test battery were used in determining domain impairment, including three tests per cognitive domain (memory, attention, executive function, and language) and one test of global cognition. Domain impairment was determined using previously described neuropsychological criteria, and was defined as a score >1.25 below norm-referenced values on two or more tests within a domain. Multiple domain impairment was assigned when more than one domain fitted the impairment criteria, or three or more tests were impaired across domains. Prior studies have established improved sensitivity and specificity of these criteria relative to those employing a single test score, as well as adaptability of this diagnostic approach to various neuropsychological batteries. Cognition was presumed normal unless multiple impaired tests were identified as specified by the criteria. Individuals with less than 15 years of education were excluded from the initial criteria. Only six participants were excluded for this reason.

Global cognition. The Mini Mental State Exam was used for UDS version 2.0 and the Montreal Cognitive Assessment for UDS version 3.0.

Memory. The Logical Memory Story A (Immediate and Delayed recall) free recall tests (modified from the original Wechsler Memory Scales—Third Edition) were used for UDS version 2.0 and the Craft Stories immediate and delayed free recall test was used for UDS version 3.0. For supplemental tests, the USC participants underwent the California Verbal Learning Test—Second Edition and the Selective Reminding Test sum of free recall trials. Norm-referenced scores for these supplemental test scores were derived from a nationally representative sample published with the test manual (California Verbal Learning Test—Second Edition) and in studies of normally aging adults (Selective Reminding Test).

Attention and executive function. The Trails A, Trails B, Wechsler Adult Intelligence Scale—Revised, and Digit Span Backwards tests were used for UDS version 2.0 and the Trials A, Trails B, and Digit Span Backwards test for UDS version 3.0.

Language. The Animal Fluency, Vegetable Fluency, and Boston Naming Tests were used for UDS version 2.0 and the Animal Fluency, Vegetable Fluency, and Multilingual Naming Test for UDS version 3.0.

VRFs. The participants' VRF burden was evaluated through physical examination, clinical blood tests, and interviews with the participant and informant; it included a history of cardiovascular disease (heart failure, angina, stent placement, coronary artery bypass graft, intermittent claudication), hypertension, hyperlipidemia, type 2 diabetes, peripheral arterial disease, transient ischemic attack or stroke. The total VRF burden was defined by the sum of these risk factors. We have previously shown that older adults with AD who exhibit two or more VRFs are more likely to exhibit occult cerebrovascular disease at autopsy, whereas a single VRF is common and not necessarily associated with increased cerebrovascular disease in this population. Thus, an elevated VRF burden was defined as an individual having two or more VRFs.

Lumbar puncture and venipuncture. Participants underwent lumbar puncture in the morning after an overnight fast. The CSF was collected in polypropylene tubes, processed (centrifuged at 2,000g, 10 min, 4°C), aliquoted into polypropylene tubes and stored at −80°C until assay. Participants underwent lumbopuncture in the morning after an overnight fast. Blood was collected into EDTA tubes and processed (centrifuged at 2,000g, 10 min, 4°C). Plasma and buffy coat were aliquoted in polypropylene tubes and stored at −80°C; buffy coat was used for DNA extraction and APOE genotyping.

APOE genotyping. DNA was extracted from buffy coat using the Quick-DNA Blood MiniPrep (Zymo Research). APOE genotyping was performed using PCR-restriction fragment length polymorphism approach. The PCR was amplified in a 50μl reaction with QIAGEN reagents (QIAQuick DNA Polymerase and 5NT Mix, PCR Grade). Two primers were used to amplify a 318-base-pair fragment: upstream primer (5’ AGTGGACCCCGGTTGCAGGAGACGCTGTCG; downstream primer (5’TGTTCACACAGGGGCCCCAGGGGCTGCGGG). The upstream primer introduces an AflIII site in the amplified product, yielding a unique restriction fragment length polymorphism pattern for each APOE allele following enzymatic digestion. The PCR reaction mixture was incubated at 94°C for 3 min, then 40 cycles of amplification (94°C, 10 s; 65°C, 30 s; 72°C, 30 s), and finally elongation at 72°C for 7 min. Restriction digestions containing 10μl ampiclons and either 2.5 U AflIII or 1.5 U HaeII were incubated at 37°C overnight. The digested products were analyzed on a 4% agarose gel. APOE genotype was determined from the unique digestion pattern: APOE2/2 (A: 231; H: 267); APOE2/3 (A: 231; H: 261 and 267); APOE2/4 (A: 231 and 295; H: 231 and 267); APOE3/3 (A: 231; H: 231); APOE3/4 (A: 231 and 295; H: 231); and APOE4/4 (A: 295; H: 231); the brackets denote base pairs of amiclons following the AflIII (A) and HaeII (H) digestions.

Molecular biomarkers in the CSF assays. Quantitative western blot analysis of sPDGF-Rβ. Quantitative western blot analysis was used to detect sPDGF-Rβ in human CSF (ng/ml−1), as we previously reported. Standard curves were generated using recombinant human PDGF-RβFc Chimera Protein (R&D Systems).

Astrocyte marker. CSF levels of the astrocytic cytokine S100B were determined using an enzyme-linked immunosorbent assay (ELISA; EMD Millipore).

Inflammatory markers. A Meso Scale Discovery (MSD) multiplex assay was used to determine CSF levels of interleukin-2 (IL-2), IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17, tumor necrosis factor-α (TNFα), and interferon γ (MSD).

Aβ peptide. An MSD multiplex assay was used to determine the CSF levels of Aβ40, Aβ42, and Aβ40. The CSF Aβ42, cutoff level of 190 pg/ml−1 was applied as previously reported for the MSD Aβ peptide assay.
Aj oligomers. CSF Aj oligomers were measured using ELISA (IBL; protocol modified from IBL and Hoftl et al. ). The Aj peptide dimer was used as the standard protein prepared at 0, 1, 2, 3, 5, 7, 10, 15, 20 μg/mL, and 100 μg/mL of prepared standards and neat CSF were added to each well on an uncoated 96-well plate along with 20 μL per well of horseradish peroxidase (HRP)-conjugated anti-human Aj (N) (82E1) mouse immunoglobulin G (IgG) monoclonal antibody; the plates were incubated overnight at 4°C on an orbital plate shaker at 600 rpm, and 100 μL per well was transferred to a 96-well plate precoated with anti-human Aj (N) (82E1) mouse IgG monoclonal antibody and incubated for 1 h at 4°C with shaking. Plates were washed with Tris-buffered saline with 0.1% Tween 20 (TBST). ELAST ELISA amplification was performed (PerkinElmer). Briefly, 100 μL per well of biotinylated tyramide (1:100 dilution) was incubated for 15 min at room temperature with shaking. Plates were washed and incubated 100 μL per well of HRP-conjugated streptavidin (1:5000 dilution) for 30 min at room temperature with shaking. Plates were washed and 100 μL per well of tetramethylbenzidine substrate (Kodak & Perry Laboratories) was incubated in the dark for 60 min, then 100 μL per well of 2 N HCl was added, and the plates were read at 450 nm.

Tau. An MSD assay was used to determine the CSF levels of total tau. Phosphorylated tau (pT181) was determined by ELISA (INNOTEST PHOSPHO- TAU(p181)) (N). The CSF pTau181 cutoff level of 78 pg/mL was applied as previously reported.

Tau oligomers. CSF tau oligomers were measured by direct ELISA using tau oligomer-specific antibody (T22). Briefly, 12 μL CSF was diluted in a total volume of 50 μL 5% BSA, 0.1% sodium carbonate buffer, pH 9.6, 0.4% Triton X-100 (TX-100), 96-well V-bottom plate (Nunc) and incubated overnight at 4°C on an orbital plate shaker at 600 rpm. Plates were washed (TBST) and blocked with 300 μL of 10% non-fat dry milk (Bio-Rad) for 2 h at room temperature with shaking. Plates were washed and incubated with 100 μL per well of T22 antibody (1:250 diluted in 5% non-fat milk) and incubated for 1 h at room temperature with shaking. Plates were washed and incubated with HRP-conjugated anti-rabbit IgG antibody (1:3,000 diluted in 5% non-fat dry milk) and incubated for 1 h at room temperature with shaking. Plates were washed and incubated in the dark with 100 μL per well tetramethylbenzidine substrate for 14 min; then, 100 μL per well 2 N HCl was added, and the plates were read at 450 nm.

Neuronal marker. CSF levels of neuron-specific enolase (NSE) were determined using ELISA (Immunologists Consultatory Laboratory).

In vitro analysis of PDGFRβ shedding. Primary human brain mural cell isolation and culture. Primary human brain vascular SMCs were isolated from leptomeningeval arteries (>100 μm diameter) as described and characterized previously. SMCs were >98% positive for α-smooth muscle actin, myosin heavy chain, calponin, and SM22 and negative for von Willebrand factor (endothelial cells), glial fibrillary acidic protein (astrocytes) and CD11b (microglia). Cells were cultured in SMC medium (ScienCell) in 5% CO2 at 37°C. Early passage (P5, P6) cultures were used in the present study.

Primary human brain microvascular pericytes were isolated from cortical brain tissue after removal of leptomeninges as described previously. Pericytes were derived from intraparenchymal microvessels that were completely free from leptomeningeval vessel contamination. Purified microvessels were largely brain capillaries (<5 μm diameter) as described and characterized previously. Cells were cultured in SMC medium (ScienCell) in 5% CO2, 37°C. Early passage (P5, P6) cultures were used in the present study.

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Treatment conditions. Primary human brain SMCs and pericytes were plated in equal cell number for all conditions. For ADAM10 knockdown, Accell Human ADAM10 (102) siRNA (Dharmacon) at a final concentration of 1 μM in Accell siRNA Delivery Media (Dharmacon) was added into 90% confluent cultured pericytes or SMCs at 12-well well plate culture plates of recommended cell density by the manufacturer, cells underwent treatment conditions. Specifically, cells were subjected to treatment with ionomycin (2.5 μM) and/or mimosatid (4 μM) prepared in reduced serum Gibco Opti-MEM (Thermo Fisher Scientific) or media only (control condition) for 40 min at 37°C, as described previously. After 40 min, the media and cell lysates were collected for additional analysis described in the next section.

Immunoprecipitation of PDGFRβ. Immunoprecipitation was performed on the pericyte and SMC media as described by the manufacturer with optimizations (all wash steps performed as described). For antibody-bead coupling, 50 μL Invitrogen Dynabeads Protein G (Thermo Fisher Scientific) and 2 μg of PDGFRβ antibody (goat antihuman; R&D Systems) were incubated with rotation for 10 min at room temperature. Conditioned media and equal volume lysis buffer were added to the Dynabeads-coupled PDGFRβ antibody and incubated with rotation for 30 min at room temperature. Target antigen was eluted in denaturing conditions and a quantitative western blot was performed as described in the next section.

Western blot analysis. A quantitative western blot on immunoprecipitated media was performed using carrier-free human recombinant PDGFRβ as a protein standard (R&D Systems). Gel transfer was performed using iBlot 2 (Thermo Fisher Scientific) at 20 V for 30 s. Cells were lysed with 0.5% Nonidet P40 (N) (82E1) mouse immunoglobulin G (IgG) monoclonal antibody; the plates were incubated overnight at 4°C on an orbital plate shaker at 600 rpm, and 100 μL per well was transferred to a 96-well plate precoated with anti-human Aj (N) (82E1) mouse IgG monoclonal antibody and incubated for 1 h at 4°C with shaking. Plates were washed with Tris-buffered saline with 0.1% Tween 20 (TBST). ELAST ELISA amplification was performed (PerkinElmer). Briefly, 100 μL per well of biotinylated tyramide (1:100 dilution) was incubated for 15 min at room temperature with shaking. Plates were washed and incubated 100 μL per well of HRP-conjugated streptavidin (1:5000 dilution) for 30 min at room temperature with shaking. Plates were washed and 100 μL per well of tetramethylbenzidine substrate (Kodak & Perry Laboratories) was incubated in the dark for 60 min, then 100 μL per well of 2 N HCl was added, and the plates were read at 450 nm.

MRI. The MRI dataset was obtained from the USC Keck Medical Center. The study was approved by the USC IRB. All participants underwent a blood draw to ensure appropriate kidney function for contrast agent administration before imaging. The imaging protocol performed was developed to detect subtle BBB changes in patients with cognitive impairment and is detailed in Montague et al. Briefly, all images were obtained on a GE 3T HDXT MR scanner with a standard eight-channel array head coil. Anatomical coronal spin echo T2-weighted scans were first obtained through the left and right HC (TR/TE 1550/97.15 ms, number of excitations = 1, slice thickness 5 mm with no gap, field of view = 188 × 180 mm², matrix size 180 × 180, voxel size 0.625 × 0.625 × 5 mm³). This sequence was repeated for a total of 16 min with an approximate time resolution of 15.4 s. The gadolinium-based contrast agents gadobenate dimeglumine (MultiHance; Bracco) or meglumine gadoterate (Dotarem; Guerbet) (0.05 mmol/kg−1) were administered intravenously into the antecubital vein using a power injector, at a rate of 3 ml/s followed by a 25 ml saline flush, 30 s into the DCE scan.

Quantification of subtle BBB permeability. Post-processing analysis was performed using ROCKETSHIP running with Matlab. The arterial input function (AIF), which was extracted from a region of interest (ROI) positioned at the internal carotid artery, was fitted with a bi-exponential function before fitting with the Patlak model. The Patlak linearized regression mathematical analysis was used to generate the BBB permeability Ktrans maps with high spatial and temporal resolutions allowing not only simultaneous measurements of the regional BBB permeability in different white and gray matter regions, but also accurate calculations of the Ktrans values in anatomical regions as small as the subdivisions of the HC. We determined in each individual AIF from the internal carotid artery. In a few cases when the common carotid artery was not clearly visible, a nearby large vessel was used. Individual AIF measurements are important particularly if the studied population diverges by age, since changes in blood volume and flow may affect AIF and the Ktrans measurements. The present analysis requires that the tracer’s diffusion across the BBB remains unidirectional during the acquisition time. The total tracer concentration in the tissue, C(t), can be described as a function of the blood concentration, C(t), and a blood-to-brain transfer constant, Ktrans, that represents the flow from the intravascular to the extravascular extracellular space using the following equation:

\[ C(t) = K_{\text{trans}} \int_{0}^{t} C(t) \, dt + C(t) A(t) \]

A statistically significant intersubject variability in the measurement of C was not observed.

ROI-averaged analysis of DCE-MRI output maps was performed by an experienced neuroradiologist who manually drew ROIs for each participant based on their own anatomy since a substantial variability between individuals is seen at a macroscopic level (for example, enlarged ventricles, cortical atrophy, hippocampal shrinkage). Thus, regional BBB Ktrans permeability was measured in 13 different gray matter ROIs including the left and right HC and their subfields (that is, CA1, CA3, and the dentate gyrus), parahippocampal gyrus (PHG), caudate nucleus, subicular, frontal cortical gyri, and the thalamus. All ROIs were defined using striatum and white matter ROIs including subcortical white matter fibers, corpus callosum, and internal capsule.

Quantification of regional brain volumes. HC and PHG morphometry were performed using the FreeSurfer (version 5.3.0) software package, which is documented and freely available online (http://surfer.nmr.mgh.harvard.edu/).
In brief, the HC and PHG were segmented using the included FreeSurfer Desikan-Killiany and subcortical atlases\textsuperscript{16,17}. Then, regional volumes (mm\textsuperscript{3}) were derived accordingly. The technical details of this procedure are described in previous publications\textsuperscript{54,55}. Data processing was performed using the Laboratory of Neuro Imaging pipeline system (http://pipeline.loni.usc.edu)\textsuperscript{42,56,57}.

PET. PiB-PET imaging was conducted at the Washington University Knight ADRC using procedures and analysis described previously\textsuperscript{54,58}.

Statistical analyses. All continuous variables were screened for outliers (\pm 3 \times \text{sd} \text{ from the mean}) and evaluated for departures from normality through quantitative examination of skewness and kurtosis, as well as visual inspection of frequency distributions. Where departures of normality were identified, log\textsubscript{10}-transformations were applied, and distribution normalization was confirmed before parametric analyses. Participant demographics and clinical characteristics were initially compared across both CDR and domain impairment stratifications using chi-squared tests and one-way analyses of variance (ANOVA), with post-hoc Tukey tests.

All CSF biomarkers were compared in parallel analyses applied across the entire sample stratified by the global CDR score and the number of impaired cognitive domains using ANCOVA, with post-hoc Bonferroni-corrected comparisons. For CDR analyses, model covariates included age, sex, education, and APOE-\varepsilon4 carrier status. For domain impairment analyses, age, sex, and education-corrected values were used to identify impairment groups and APOE-\varepsilon4 carrier status was used as a covariate. Site-specific analyses and interaction effect analyses did not include APOE-\varepsilon4 carrier status as a covariate to conserve statistical power. For the analysis of interactions by A\beta, pTau, and VRF burden, statistical interactions and main effects were examined in similar ANCOVA models.

The same approach described was used in all analyses of other CSF glial, inflammatory, and neuronal markers, and for the DCE-MRI data. With regard to missing data, all participants had complete data for primary outcomes (CSF sPDGFR\beta and DCE-MRI); the extent of missing data was capped at \textless 10\% for all other CSF biomarkers and clinical measures (that is, \textgreater 90\% of participants had complete data).

Given the large number of analyses, false discovery rate (FDR) correction was applied to all ANCOVA omnibus \textit{P} values using the Benjamini–Hochberg method\textsuperscript{59}.

Where significant CSF sPDGFR\beta and BBB \textit{K}_{\text{trans}} findings were identified (CDR 0.5 versus 0 and domain impairment 1+ versus 0), separate post-hoc analyses of CSF sPDGFR\beta and BBB \textit{K}_{\text{trans}} differences controlling for CSF A\beta, pTau, PiB-PET amyloid deposition, pTau oligomers, and HC and PHG volumes also utilized ANCOVA models. In addition, separate hierarchical logistic regression analyses evaluated whether CSF sPDGFR\beta and BBB \textit{K}_{\text{trans}} predicted cognitive impairment (CDR 0.5 versus 0 and domain impairment 1+ versus 0) after controlling for CSF A\beta, pTau, PiB-PET amyloid deposition, pTau oligomers, and HC and PHG volumes. For both ANCOVA and logistic regression analyses, covariates were entered into the model in the first block; in the second block, either CSF sPDGFR\beta or specific regional BBB \textit{K}_{\text{trans}} values were entered. Additional demographics and APOE-\varepsilon4 carrier status were included in the overall models correcting for CSF A\beta, pTau, and models correcting for HC and PHG volumes.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data included in this study are available in Supplementary Tables containing detailed statistical analyses and in the accompanying Source Data. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | ADAM10 mediates sPDGFRβ shedding in human brain pericytes in vitro. 

**a**, Primary human brain vascular SMCs and pericytes were subjected to treatment with ionomycin (2.5 μM), a calcium ionophore that activates ADAM10, or control treatment (media only); media was immunoprecipitated to measure sPDGFRβ by quantitative western blot. Compared to pericytes, SMCs shed extremely low levels of sPDGFRβ, which was not significantly increased by ionomycin. Pericytes shed high basal levels of sPDGFRβ that was significantly increased fivefold by treatment with ionomycin, which activated ADAM10. To further determine the involvement of ADAM10, ionomycin treatment was conducted in the presence of the pharmacological inhibition of ADAM10 with marimastat (4 μM), which inhibits ADAM10 by binding to active-site zinc, and genetic small interfering RNA (siRNA) knockdown of ADAM10. Both pharmacological (marimastat) and genetic (siRNA) inhibition of ADAM10 significantly reduced sPDGFRβ shedding activated by ionomycin by >90 and 75%, respectively. 

**b**, The siRNA ADAM10 knockdown efficiency in this study was 85%, as shown by the western blot analysis. Data was generated from $n=3–6$ independent culture experiments and plotted as means ± s.e.m. 

**a**, SMC data by two-tailed Student’s t-test; pericyte data by ANOVA with Tukey post-hoc test. 

**b**, Two-tailed Student’s t-test. Significance set at $\alpha = 0.05$ for all analyses.
Extended Data Fig. 2 | CSF sPDGFRβ increases with CDR impairment, independent of Aβ and tau, and reflects BBB breakdown. a–b, Site-specific analysis of CSF sPDGFRβ and the standard AD biomarkers, Aβ\textsubscript{42} and ptau, indicates an early increase in sPDGFRβ with increasing CDR at both independent clinical sites, USC (a) and Washington University (b). There were no changes in Aβ\textsubscript{42} and ptau at the USC site (a), whereas Aβ\textsubscript{42} but not ptau, was altered at the Washington University site; supports Fig. 1a–c. c–d, Site-specific analysis of CSF sPDGFRβ increases with CDR, independent of CSF Aβ\textsubscript{42} and ptau status at two independent sites, USC (c) and Washington University (d); supports Fig. 1d–f. e–f, CSF sPDGFRβ is associated with BBB breakdown. CSF sPDGFRβ positively correlates with the conventional biochemical biomarkers of BBB breakdown including the CSF/plasma albumin ratio (Q\textsubscript{alb}) (e) and CSF fibrinogen (f); supports Figs. 1 and 3. g, h, BBB breakdown is independent of amyloid and tau oligomers. No differences were observed in CSF Aβ\textsubscript{42} (h) and tau (i) oligomer levels in individuals with CDR 0 versus CDR 0.5; supports Fig. 1d–f. j–k, Increases in CSF sPDGFRβ (j) and regional BBB K\textsubscript{trans} in the HC (k) and PHG (k) of individuals with CDR 0.5 versus CDR 0 remain significant after statistically controlling for the impact of CSF tau oligomers; supports Fig. 1d–f. a–d, g–i, The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. a–d, g–i, Significance tests from ANCOVAs. e–f, Statistical significance determined by Pearson correlation coefficient (r). h–i, Significance by two-tailed Student’s t-test at α = 0.05. j–k, ANCOVA models representing estimated marginal means ± s.e.m. The brackets denote the sample size (n) in each analysis.
Extended Data Fig. 3 | sPDGFRβ increases with CDR independent of VRFs, and no change in other neurovascular unit biomarkers. a–c. CSF sPDGFRβ is increased with CDR, independent of VRF burden in the combined site analysis (a) and at two independent clinical sites from USC (b) and Washington University (c). VRFs 0–1: no or 1 VRF. VRFs 2+: 2 or more VRFs. See Supplementary Table 1 for the list of VRFs; supports Fig. 1a–f. The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. Significance tests from ANCOVAs. The brackets denote the sample size (n) in each analysis.
Extended Data Fig. 4 | Other CSF biomarkers of the neurovascular unit are not altered with CDR cognitive impairment. a–c. CSF markers of glial, inflammatory, or neuronal injury exhibited no significant differences between unimpaired and impaired individuals on CDR, including S100B, IL-6, TNFα, or NSE in the combined site analysis (a) and similarly in the site-specific analysis of individuals from USC (b) and Washington University (c); supports Fig. 1a–c. The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. Significance tests from ANCOVAs. The brackets denote the sample size (n) in each analysis.
Extended Data Fig. 5 | Regional BBB breakdown $K_{\text{trans}}$ increases with CDR independent of CSF Aβ, tau, and VRFs, and relates to sPDGFRβ only in hippocampal gray matter regions. a–b, An increase in $K_{\text{trans}}$ values in the HC, PHG, and the CA1, CA3, and dentate gyrus HC subfields, with increasing CDR (a), but not in other brain regions including the superior frontal cortical gyrus and inferior temporal cortical gyrus, white matter regions including the subcortical white matter fibers, corpus callosum, and internal capsule, and deep gray matter regions including the thalamus, caudate nucleus, and striatum (b). c–d, Additional brain regions showed no significant differences in $K_{\text{trans}}$ BBB permeability values in individuals with CDR 0 and CDR 0.5, regardless of CSF Aβ or pTau status. e–f, VRF burden does not influence an increase in the $K_{\text{trans}}$ BBB permeability values with increasing CDR in the HC, PHG, and HC subfields (that is, CA1, CA3, and dentate gyrus) (e), and no change in the $K_{\text{trans}}$ BBB permeability values in other brain regions (f). See Supplementary Table 1 for the list of VRFs. a–f, Supports Fig. 1g–k. g–j, CSF sPDGFRβ is associated with BBB breakdown measured by neuroimaging in hippocampal gray matter regions (g–h), but not in white matter regions (i–j); supports Figs. 1 and 3. a–f, The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. Significance tests after FDR correction from ANCOVAs. g–j, Statistical significance determined by Pearson correlation coefficient ($r$). The brackets denote the sample size ($n$) in each analysis; applies to all regions within each panel.
Extended Data Fig. 6 | CSF sPDGFRβ increases with CDR impairment, independent of Aβ, tau, and VRFs. a–b, Site-specific analysis of CSF sPDGFRβ and the standard AD biomarkers, Aβ42 and pTau, indicates an early increase in sPDGFRβ with increasing impaired domains at both independent clinical sites, USC (a) and Washington University (b); supports Fig. 3a–c. c–d, Site-specific analysis of CSF sPDGFRβ indicates increases with the number of impaired cognitive domains, independent of CSF Aβ42 and pTau status at two independent sites, USC (c) and Washington University (d); supports Fig. 3d–f. e–g, CSF sPDGFRβ is increased with the increasing number of impaired cognitive domains, independent of VRF burden in the combined site analysis (e) and at two independent clinical sites, USC (f) and Washington University (g). VRFs 0–1: no or 1 VRF. VRFs 2+: 2 or more VRFs. See Supplementary Table 2 for the list of VRFs. Supports Fig. 3a–f, a–g. The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. Significance tests from ANCOVAs. The brackets denote the sample size (n) in each analysis.
Extended Data Fig. 7 | BBB breakdown is independent of amyloid and tau oligomers. a, CSF sPDGFRβ is increased with impaired cognitive domains, independent of amyloid positivity by PiB-PET, supports Fig. 3d-f. b–c, No differences were observed in CSF Aβ (b) and tau (c) oligomer levels in individuals with 0 or 1+ impaired cognitive domains. d–e, Increases in CSF sPDGFRβ (d) and regional BBB K_trans in the HC and PHG (e) of individuals with 1+ versus 0 cognitive domain impairment remain significant after statistically controlling for the impact of CSF tau oligomers; supports Fig. 3d-f. a–c, The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. a, Significance tests from ANCOVAs. b–c, Significance by two-tailed Student’s t-test at α = 0.05. d–e, ANCOVA models representing the estimated marginal means ± s.e.m. The brackets denote the sample size (n) in each analysis.
Extended Data Fig. 8 | Other CSF biomarkers of the neurovascular unit are not altered with cognitive domain impairment. a–c. CSF markers of glial, inflammatory, or neuronal injury exhibited no significant differences between unimpaired and impaired individuals on neuropsychological exams, including S100B, IL-6*, TNFα†, or NSE† in the combined site analysis (a) or in the site-specific analysis of individuals from USC (b) or from Washington University (c). a–c. The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. Significance tests after FDR correction from ANCOVAs with post-hoc Bonferroni comparisons. The brackets denote the sample size (n) in each analysis. *Analysis did not survive significance after FDR correction. †Individual group comparison P values reported because the omnibus test was P < 0.05 but the post-hoc group comparisons were null. Supports Fig. 3a–c.
Extended Data Fig. 9 | Regional BBB breakdown $K_{trans}$ increases with cognitive domain impairment, independent of CSF Aβ, tau, and VRFs. 

a–b, An increase in $K_{trans}$ values in the HC, PHG, and the CA1, CA3, and dentate gyrus HC subfields with increasing cognitive impairment measured by the number of impaired cognitive domains (a), but not in other brain regions, including the superior frontal cortical gyrus and the inferior temporal cortical gyrus, white matter regions including the subcortical white matter fibers, corpus callosum, and internal capsule, and deep gray matter regions including thalamus, caudate nucleus, and striatum (b). c–d, Additional brain regions showed no significant difference in $K_{trans}$ BBB permeability in individuals with 0 and 1+ impaired cognitive domains, regardless of CSF Aβ42 (c) and ptau (d) status. 

e–f, $K_{trans}$ BBB permeability is increased with increasing cognitive domain impairment in the HC, PHG, and HC subfields (that is, CA1, CA3, and dentate gyrus), independent of VRF burden (e), but not in other brain regions (f). VRFs 0–1: no or 1 VRF; VRFs 2+: 2 or more VRFs. See Supplementary Table 2 for the list of VRFs. a–f, The box and whisker plot lines indicate the median values, the boxes indicate the IQR, and the whiskers indicate the minimum and maximum values. Significance tests after FDR correction from ANCOVAs. The brackets denote the sample size (n) in each analysis; applies to all regions within each panel. Supports Fig. 3g–k.
Extended Data Fig. 10 | CSF sPDGFRβ and medial temporal BBB permeability $K_{\text{trans}}$ values are not correlated with age, indicating that changes in CSF sPDGFRβ and $K_{\text{trans}}$ capture processes relating to cognitive impairment are independent of normal aging. In CDR 0 individuals, age does not correlate with CSF sPDGFRβ (a) or regional $K_{\text{trans}}$ in the HC (c) and PHG (e). Similarly, in CDR 0.5 individuals, age does not correlate with CSF sPDGFRβ (a) or regional $K_{\text{trans}}$ in the HC (c) and PHG (e). Statistical significance determined by Pearson correlation coefficient ($r$); the brackets denote the sample size ($n$) in each analysis. Supports Figs. 1 and 3.
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| Give P values as exact values whenever suitable. | Yes       |
| For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings | Yes       |
| For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes | Yes       |
| Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated | Yes       |
| Clearly defined error bars | Yes       |
| State explicitly what error bars represent (e.g. SD, SE, CI) | Yes       

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Our GUI code (https://github.com/petmri/ROCKETSHIP) running with Matlab R2013a was used for DCE-MRI analyses. We also used FreeSurfer (v5.3.0) software package for regional brain volume analyses.

Data analysis

Statistical analyses were conducted with a commercial statistical software package -- SPSS (IBM). No custom software was used.

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A full data availability statement is included in the manuscript. The data that support the findings of this study are available from the corresponding author upon reasonable request.
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- Life sciences
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Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

We expected a sample size of n>150 to be sufficient to detect significant blood-brain barrier marker effects on cognitive status based on observed effect sizes reported in prior studies (see Montagne et al., Neuron). Consistent with previous studies, we observed large effect sizes for our primary analyses in the present study (up to $\eta^2 > .32$, indicating >99% power to detect significant differences, $\lambda = 47.04$, for an $\alpha = .05$ in a post-hoc power analysis). On the lowest end, we observed a smallest effect size of $\eta^2 = .07$, indicating 90% power, $\lambda = 10.53$. For DCE-MRI $k_{trans}$ markers of blood-brain barrier breakdown, we observed similar effect sizes, indicating >99% power ($\lambda = 22.89$) to detect significant differences for large effects $\eta^2 > .32$ with 73 participants, and ~70% power ($\lambda = 6.57$) for smaller effect sizes, $\eta^2 = .09$. Post-hoc power analyses were conducted using G*Power.

Data exclusions

All continuous variables were screened for outliers (+/- 3 SDs from mean were removed based on pre-established criteria) and evaluated for departures from normality through quantitative examination of skewness and kurtosis, as well as visual inspection of frequency distributions. Further, as stated in our Inclusion / Exclusion criteria (see Online Methods), we excluded participants with any history of psychiatric or neurological disease, or taking any medications, that might better account for observed cognitive impairment. The text from Online Methods is provided below: Included participants (≥45 years of age) with neuropsychologically-confirmed no cognitive dysfunction and/or early cognitive dysfunction had no current or prior history of any neurological or psychiatric conditions that might better account for any observed cognitive impairment, including organ failure, brain tumors, epilepsy, hydrocephalus, schizophrenia, major depression. Participants were stratified based on CSF analysis as either $A\beta$-positive ($A\beta^+$, <190 pg/mL) or $A\beta$-negative ($A\beta^-$, >190 pg/mL), or pTau-positive (pTau+, >78 pg/mL) or pTau-negative (pTau-, <78 pg/mL), using the accepted cutoff values. Participants were excluded if they were diagnosed with vascular cognitive impairment or vascular dementia. These clinical diagnoses were conducted by neurologists and the criteria whether the patient 1) had a known vascular brain injury and 2) the clinician judged that the vascular brain injury played a role in their cognitive impairment, and/or pattern and course of symptoms. In addition to clinical diagnosis, presence of vascular lesions was confirmed by moderate-to-severe white matter changes and lacunar infarcts by fluid-attenuated inversion recovery (FLAIR) MRI and/or subcortical microbleeds by T2*-weighted MRI. Participants were also excluded if they were diagnosed with Parkinson’s disease, Lewy body dementia or frontotemporal dementia. History of a single stroke or transient ischemic attack was not an exclusion unless it was related to symptomatic onset of cognitive impairment. Participants also did not have current contraindications to MRI and were not currently using medications that might better account for any observed cognitive impairment.

Replication

All study findings regarding CSF biomarkers analyses were replicated in two independent samples, as shown in the supplemental figures. The DCE-MRI findings were conducted only at one site due to limited availability of this method at the time of the current study--future studies will examine DCE-MRI across sites.

Randomization

We did not randomize since this was not an experimental design.

Blinding

All CSF assays and DCE-MRI scans were conducted by investigators who were blinded to participant clinical diagnostic status.

Materials & experimental systems

Policy information about availability of materials

n/a | Involved in the study
---|---
| Unique materials
| Antibodies
| Eukaryotic cell lines
| Research animals
| Human research participants

Antibodies

For quantitative western blot assay, the following primary antibody was used: PDGFRbeta polyclonal goat IgG antibody (R&D Systems Catalog #AF1042, Lot #G00418041). Membranes were incubated with 1 ug/mL of antibody overnight at room temperature, then incubated with donkey anti-goat IgG secondary antibody (Invitrogen, Cat #A15999, Lot #44-33-100114, 1:5000 dilution) for 1 hour at room temperature.
Validation

Recombinant human PDGFRbeta protein (R&D Systems Catalog #385-PR/CF, Lot #AMO0714072) was used as a positive control in validating the antibodies. Consistently, the manufacturer's website specifies this antibody exhibits approximately 35% cross-reactivity with recombinant human PDGFRbeta.

Human research participants

Policy information about studies involving human research participants

Population characteristics

We studied 161 participants with clinical dementia ratings of 0 (n=82), 0.5 (n=63), or 1 (n=16), who exhibited no cognitive domain impairment (n=83), one impaired domain (n=39) or two or more impaired domains (n=39). Relevant covariates (means +/- standard deviations) included age (72.3 +/- 9.6 years), sex (51.6% male), education (15.5 +/- 9.6 years) and APOE4 carrier status (44.5%).

Method-specific reporting

n/a

Involved in the study

- ChIP-seq
- Flow cytometry
- Magnetic resonance imaging

Magnetic resonance imaging

Experimental design

Design type

Resting state

Design specifications

N/A

Behavioral performance measures

N/A

Acquisition

Imaging type(s)

Dynamic Contrast-Enhanced (DCE)-MRI

Field strength

3T

Sequence & imaging parameters

From Online Methods: Anatomical coronal spin echo T2-weighted scans were first obtained through the hippocampi (TR/TE 1550/97.15 ms, NEX = 1, slice thickness 5 mm with no gap, FOV = 188 x 180 mm, matrix size = 384 x 384). Baseline coronal T1-weighted maps were then acquired using a T1-weighted 3D spoiled gradient echo (SPGR) pulse sequence and variable flip angle method using flip angles of 2°, 5° and 10°. Coronal dynamic contrast-enhanced (DCE)-MRI covering the hippocampi and temporal lobes were acquired using a T1-weighted 3D SPGR pulse sequence (FA = 15°, TR/TE = 8.29/3.09 ms, NEX = 1, slice thickness 5 mm with no gap, FOV 188 x 180 mm, matrix size 160 x 160, voxel size was 0.625 x 0.625 x 5 mm3). This sequence was repeated for a total of 16 min with an approximate time resolution of 15.4 s. Gadolinium-based CA, Gadobenate dimeglumine (MultiHance®, Bracco, Princeton, New Jersey) or Gadoterate meglumine (Dotarem®, Guerbet, France) (0.05 mmol/kg) was administered intravenously into the antecubital vein using a power injector, at a rate of 3 mL/s followed by a 25 mL saline flush, 30 s into the DCE scan.

Area of acquisition

Scan was coronal with slices covering hippocampi, temporal lobes and other in plane regions. These regions were chosen due to their importance in aging, cognitive decline, Alzheimer's disease, and prior studies of blood-brain barrier dysfunction in older adults.

Diffusion MRI

- Used
- Not used

Preprocessing

Preprocessing software

From Online Methods:

- Blood-brain barrier permeability: Post-processing analysis was performed using Rocketship71 running with Matlab. The arterial input function (AIF), which was extracted from a region-of-interest (ROI) positioned at the internal carotid artery, was fitted with a bi-exponential function prior to fitting with Patlak model72. The Patlak linearized regression mathematical analysis was used to generate the BBB permeability Ktrans maps8,71,72 with high spatial and temporal resolutions allowing not only simultaneous measurements of the regional BBB permeability in different white (WM) and gray matter (GM) regions, but also accurate calculations of the Ktrans values in anatomical regions as small as the subdivisions of the hippocampus. We determined in each individual AIF from the internal carotid artery.
- Volumetric analysis: Hippocampus (HC) and parahippocampus (PHC) morphometry were performed using the FreeSurfer (v5.3.0) software package73, which is documented and freely available online (http://surfer.nmr.mgh.harvard.edu/). In brief, HC and PHC gyri were segmented using the included FreeSurfer Desikan-Killiany and subcortical atlases74,75. Then, regional volumes (mm3) were derived accordingly. The technical details of this procedure are described in previous publications76–79. Data processing was performed using the Laboratory of Neuro Imaging (LONI) pipeline system (http://pipeline.loni.usc.edu)80–84.

Normalization

N/A
**Normalization template**

N/A

**Noise and artifact removal**

Motion correction (for DCE-MRI) was applied using ImageJ's Stack Reg - Rigid Body plugin.

**Volume censoring**

N/A

### Statistical modeling & inference

#### Model type and settings

N/A

#### Effect(s) tested

N/A

#### Specify type of analysis:

- [ ] Whole brain
- [x] ROI-based
- [ ] Both

**Anatomical location(s)**

The regional BBB K\textsubscript{trans} permeability were measured in 13 different GM ROIs including the hippocampi [HC] and their subfields (i.e., CA1, CA3, and dentate gyrus [DG]), parahippocampus [PHC], caudate nucleus [Caud], superior frontal cortical gyri [SFG Cx], inferior temporal cortical gyr [ITG Cx], thalamus [Thal], and striatum [Str] and WM ROIs including subcortical frontal white matter fibers [SubP WM fibers], corpus callosum (CC), and internal capsule (IC). Regions were chosen to cover a variety of brain regions and tissue compartments (e.g., cortical white matter, subcortical white matter, cortical grey matter, subcortical grey matter, and limbic areas of special focus—in this case the hippocampus and medial temporal lobes). The regional brain volumes were measured in HC and PHC gyri.

**Statistic type for inference**

(See Eklund et al. 2016)

We did not conduct a voxel-level analysis.

**Correction**

We did not conduct a voxel-level analysis.

### Models & analysis

- [x] Involved in the study

- [ ] Functional and/or effective connectivity
- [x] Graph analysis
- [x] Multivariate modeling or predictive analysis