Vascular contributions to dementia and Alzheimer’s disease are increasingly recognized. Recent studies have suggested that breakdown of the blood–brain barrier (BBB) is an early biomarker of human cognitive dysfunction, including the early clinical stages of Alzheimer’s disease. The E4 variant of apolipoprotein E (APOE4), the main susceptibility gene for Alzheimer’s disease, leads to accelerated breakdown of the BBB and degeneration of brain capillary pericytes, which maintain BBB integrity. It is unclear, however, whether the cerebrovascular effects of APOE4 contribute to cognitive impairment. Here we show that individuals bearing APOE4 (with the ε3/ε4 or ε4/ε4 alleles) are distinguished from those without APOE4 (ε3/ε3) by breakdown of the BBB in the hippocampus and medial temporal lobe. This finding is apparent in cognitively unimpaired APOE4 carriers and more severe in those with cognitive impairment, but is not related to amyloid-β or tau pathology measured in cerebrospinal fluid or by positron emission tomography. High baseline levels of the BBB pericyte injury biomarker soluble PDGFRβ in the cerebrospinal fluid predicted future cognitive decline in APOE4 carriers but not in non-carriers, even after controlling for amyloid-β and tau status, and were correlated with increased activity of the BBB-degrading cyclophilin A-matrix metalloproteinase-9 pathway in cerebrospinal fluid. Our findings suggest that breakdown of the BBB contributes to APOE4-associated cognitive decline independently of Alzheimer’s disease pathology, and might be a therapeutic target in APOE4 carriers.
These findings held when cognitive dysfunction was evaluated by neuropsychological performance (see Methods) (Extended Data Figs. 2, 3).

The volumes of the HC and PHG decreased with cognitive impairment in APOE4 but not APOE3 carriers (Fig. 1f–k). The breakdown of the BBB in the HC and PHG in APOE4 carriers, but not APOE3 homozygotes, remained a highly significant predictor of cognitive impairment after we statistically controlled for age, sex, education, CSF Aβ1–42 and pTau statuses, and HC and PHG volumes, as shown by the estimated marginal means from the ANCOVA models (Supplementary Table 1). The BBB dysfunction (Fig. 1c, d, i, m) preceded brain atrophy (Fig. 1j, k) and was independent of systemic vascular risk factors (Extended Data Fig. 4).

Because both Aβ and tau can lead to blood vessel abnormalities and BBB breakdown, we studied whether BBB disruption in APOE4 carriers was downstream from amyloid and tau accumulation in a subset of 74 and 96 participants, respectively (Extended Data Tables 2a, b). Voxel-based analysis by positron emission tomography (PET) indicated a substantially higher accumulation of amyloid in the orbital frontal cortex (OFC) in cognitively normal APOE4 carriers compared to APOE3 homozygotes, as reported, but did not detect accumulation of tau tracer in either APOE4 or APOE3 carriers (Extended Data Fig. 5a–d). To determine how BBB permeability relates to accumulation of amyloid and tau, we selected 5-mm-thick coronal slices in regions of interest that included the HC and PHG (where amyloid disruption is seen first in APOE4 carriers, compared to APOE3 carriers) (Extended Data Fig. 3, b, d). We used a substantially higher accumulation of amyloid in the orbital frontal cortex (OFC) in cognitively normal APOE4 carriers compared to APOE3 homozygotes, as reported, but did not detect accumulation of tau tracer in either APOE4 or APOE3 carriers (Extended Data Fig. 5a–d). To determine how BBB permeability relates to accumulation of amyloid and tau, we selected 5-mm-thick coronal slices in regions of interest that included the HC and PHG (where amyloid disruption is seen first in APOE4 carriers, compared to APOE3 carriers) (Extended Data Fig. 3, b, d). We used a substantially higher accumulation of amyloid in the orbital frontal cortex (OFC) in cognitively normal APOE4 carriers compared to APOE3 homozygotes, as reported, but did not detect accumulation of tau tracer in either APOE4 or APOE3 carriers (Extended Data Fig. 5a–d). To determine how BBB permeability relates to accumulation of amyloid and tau, we selected 5-mm-thick coronal slices in regions of interest that included the HC and PHG (where amyloid disruption is seen first in APOE4 carriers, compared to APOE3 carriers) (Extended Data Fig. 3, b, d). We used a substantially higher accumulation of amyloid in the orbital frontal cortex (OFC) in cognitively normal APOE4 carriers compared to APOE3 homozygotes, as reported, but did not detect accumulation of tau tracer in either APOE4 or APOE3 carriers (Extended Data Fig. 5a–d). To determine how BBB permeability relates to accumulation of amyloid and tau, we selected 5-mm-thick coronal slices in regions of interest that included the HC and PHG (where amyloid disruption is seen first in APOE4 carriers, compared to APOE3 carriers) (Extended Data Fig. 3, b, d).
Extended Data Fig. 5f, g) indicated no difference between APOE4 and APOE3 carriers in the HC, although uptake of both tracers was modestly increased compared to the background values in cerebellum (Fig. 2a, b). The BBB was disrupted in the HC in APOE4 carriers compared to APOE3 homozygotes (Fig. 2c), consistent with our findings in the larger cohort (Fig. 1b, c). There was no difference in amyloid and tau accumulation in the PHG between APOE3 homozygotes, despite BBB disruption in APOE4 carriers (Fig. 2d–f). Amyloid accumulation in the PHG was higher in cognitively normal APOE4 carriers than in APOE3 carriers (Fig. 2g, h), but there was no difference in BBB integrity (Fig. 2g, i). In the ITG, there were no differences in tau accumulation or BBB integrity between APOE4 and APOE3 carriers (Fig. 2j–l). Together, these data suggest that BBB disruption in the HC and PHG in APOE4 carriers is independent of AD pathology, and that BBB breakdown in APOE4 carriers starts in the medial temporal lobe, which is responsible for memory encoding and other cognitive functions.

In humans with AD and animal models, elevated levels of soluble platelet-derived growth factor receptor-β (sPDGFRβ) in the CSF indicate that pericyte injury is linked to BBB breakdown and cognitive dysfunction. Using a median split for visual display of the CSF sPDGFRβ baseline levels from 350 participants (see Methods), we stratified all participants into two groups, with low CSF sPDGFRβ levels (0–600 ng ml⁻¹) and high sPDGFRβ levels (600–2,000 ng ml⁻¹) (Fig. 3a). In 146 APOE4 carriers and APOE3 homozygotes who were evaluated...
by cognitive exams at 2-year intervals up to 4.5 years from baseline, participants with higher CSF sPDGFRβ exhibited accelerated cognitive decline on a global mental status exam and global cognitive composite z-scores, which remained significant after controlling for CSF Aβ and tau status (Fig. 3b, c; Supplementary Table 2). When stratified by APOE status, higher baseline CSF sPDGFRβ levels in APOE4 carriers predicted cognitive decline after controlling for CSF Aβ (P = 0.002) and pTau (P = 0.002) status; b, and in global cognitive composite scores (P = 0.01) (this remains significant after controlling for CSF Aβ (P = 0.017) and pTau (P = 0.01) status; c, d, e, Higher CSF sPDGFRβ (blue) in APOE4 carriers (n = 58) predicts future decline in mental status exam scores (P = 0.005) after controlling for CSF Aβ (P = 0.004) and pTau (P = 0.003) status (d), and in global cognitive composite scores (P = 0.02) after controlling for CSF Aβ (P = 0.02) and pTau (P = 0.01) status (e). f, g, Baseline CSF sPDGFRβ does not predict decline (n = 88) in either mental status (f) or global composite (g) scores in APOE3 homozygotes regardless of CSF Aβ or pTau status.

Brain tissue analysis has also shown higher activation of the CypA–MMP9 pathway in degenerating brain capillary pericytes in APOE4 carriers than in APOE3 homozygotes. In our cohort, APOE4 carriers, but not APOE3 homozygotes, developed an increase in CypA CSF levels with cognitive impairment (Fig. 4h, i), which correlated with elevated CSF sPDGFRβ (Fig. 4j). APOE4 carriers, but not APOE3 homozygotes, also developed elevated MMP9 in the CSF with cognitive impairment (Fig. 4k), which correlated with elevated CSF CypA levels (Fig. 4i), suggesting that activation of the CypA–MMP9 pathway in APOE4 carriers correlates with pericyte injury, as shown in animal models. There were no differences in glia or inflammatory or endothelial cell injury CSF biomarkers between cognitively impaired and unimpaired APOE4 and APOE3 participants, but there was an increase in neuron-specific enolase (NSE) with cognitive impairment in APOE4 carriers, confirming neuronal stress (Extended Data Fig. 6) and consistent with atrophy of the HC and PHG (Fig. 1j, k).

Studies in APOE knock-in mice and mouse pericytes have shown that apoE3, but not apoE4, transcriptionally inhibits CypA via low-density lipoprotein receptor-related protein 1, which in turn transcriptionally inhibits MMP9. Consistent with the mouse data, pericytes derived from APOE4 (ε4/ε4) human induced pluripotent stem cells (iPSCs) had substantially higher levels of CypA and secreted MMP9 than those derived from APOE3 (ε3/ε3) cells (Fig. 4m, n), suggesting that...
apoE may control the CypA-MMP9 pathway in human pericytes in an isoform-specific manner, as in mouse models\(^6\).

In APOE4 carriers, CSF Aβ\(_{1-42}\) was reduced and CSF pTau levels were increased with cognitive impairment, compared to APOE3 homozygotes (Extended Data Fig. 7), as reported\(^6\); this difference remained significant after controlling for CSF sPDGFRβ and albumin quotient (Q\(_{\text{albumin}}\) = 92); fibrinogen (n = 93); and plasminogen (n = 72) in APOE4 carriers. e, g, Correlations between CSF sPDGFRβ and albumin quotient (Q\(_{\text{albumin}}\)); fibrinogen (n = 93); and plasminogen (n = 72) in APOE4 carriers. h, CSF CypA in individuals with CDR 0 bearing APOE3 (black, n = 75) or APOE4 (red, n = 62) and with CDR 0.5 bearing APOE3 (black, n = 33) or APOE4 (red, n = 45). j, Correlation between CSF CypA and sPDGFRβ in APOE4 carriers (n = 96). k, CSF matrix metalloproteinase-9 (MMP9) in individuals with CDR 0 bearing APOE3 (black, n = 72) or APOE4 (red, n = 68) and with CDR 0.5 bearing APOE3 (black, n = 33) or APOE4 (red, n = 45). l, Correlation between CSF MMP9 and CypA in APOE4 carriers (n = 104). m, n, CypA (m; see Extended Data Fig. 8) and secreted MMP9 in the culture medium (n) in human iPSC-derived APOE3 (ε3/ε3) and APOE4 (ε4/ε4) pericytes. Mean ± s.e.m. from four independent culture replicates. a, b, Continuous lines, median; dotted lines, IQR. Significance by ANCOVA for main effects and post hoc comparisons controlling for age, sex, and education. c, g, j, Two-tailed simple linear regression; Pearson correlation coefficient (r). m, n, Unpaired two-tailed Student’s t-test.

In summary, we have shown that BBB breakdown contributes to cognitive decline in APOE4 carriers independent of AD pathology; that high baseline CSF levels of sPDGFRβ can predict future cognitive decline in APOE4 carriers; and that APOE4, but not APOE3, activates the CypA-MMP9 pathway in the CSF, which may lead to accelerated BBB breakdown and thereby cause neuronal and synaptic dysfunction\(^6\). As blockade of the CypA-MMP9 pathway in APOE4 knock-in mice restores BBB integrity and subsequently normalizes neuronal and synaptic function\(^6\), it is possible that CypA inhibitors (some of which have been used...
in humans for non-neurological applications\(^3\) might also suppress the CypA pathway in cerebral blood vessels in \(\text{APOE4} \) carriers. This should improve cerebrovascular integrity, and reduce the associated neuronal and synaptic deficits, thereby slowing cognitive impairment.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2247-3.
Methods

Study participants
Participants were recruited from three sites: the University of Southern California (USC), Los Angeles, CA; Washington University (WashU), St. Louis, MO; and Banner Alzheimer’s Institute Phoenix, AZ and Mayo Clinic Arizona, Scottsdale, AZ as a single site. At the USC site, participants were recruited through the USC Alzheimer’s Disease Research Center (ADRC); combined USC and the Huntington Medical Research Institutes (HMRI), Pasadena, CA. At the WashU site, participants were recruited through the Washington University Knight ADRC. At Banner Alzheimer’s Institute and Mayo Clinic Arizona site, participants were recruited through the Arizona Apolipoprotein E (APOE) cohort. The study and procedures were approved by the Institutional Review Boards of USC ADRC, Washington University Knight ADRC, and Banner Good Samaritan Medical Center and Mayo Clinic Scottsdale, indicating compliance with all ethical regulations. Informed consent was obtained from all participants before study enrolment. All participants (n = 435) underwent neurological and neuropsychological evaluations performed using the Uniform Data Set (UDS) and additional neuropsychological tests, as described below, and received a venipuncture for collection of blood for biomarker studies. An LP was performed in 350 participants (81%) for collection of CSF. DCE-MRI for assessment of BBB permeability was performed in 245 participants (56%) who had no contraindications for contrast injection. Both LP and DCE-MRI were conducted in 172 participants. Among the 245 DCE-MRI participants, 74 and 96 were additionally studied for brain uptake of amyloid and tau PET radiotracers, respectively, as described below. No statistical methods were used to predetermine sample size. All biomarker assays, MRI, and PET scans were analysed by investigators blinded to the clinical status of the participants.

Participant inclusion and exclusion criteria
Included participants (≥45 years of age) were confirmed by clinical and cognitive assessments to be either cognitively normal or at the earliest symptomatic stage of AD. A current or prior history of any neurological or psychiatric conditions that might confound cognitive assessment, including organ failure, brain tumors, epilepsy, hydrocephalus, schizophrenia, and major depression, was exclusionary. Participants were stratified by APOE genotype as APOE4 carriers (ε3/ε4 and ε4/ε4) or APOE4 non-carriers (ε3/ε3), also defined as APOE3 homozygotes, who were cognitively normal or had mild cognitive dysfunction, as determined by CDR scores and the presence of cognitive impairment in one or more cognitive domains based on comprehensive neuropsychological evaluation, including performance on ten neuropsychological tests assessing memory, attention/executive function, language and global cognition. For all analyses individuals with ε3/ε4 and ε4/ε4 alleles were pooled together in a single APOE4 group, as we did not find in the present cohort (82–86% ε3/ε4 and 14–18% ε4/ε4 participants, depending on the outcome measure) a significant difference between individuals with two versus one ε4 allele for the studied parameters, including the BBBKtrans and sPDGFRβ CSF values (see statistical section below). Individuals were additionally stratified by Aβ and pTau CSF analysis as either Aβ1–42+ (<190 pg/ml) or Aβ1–42− (<190 pg/ml), and pTau+ (>78 pg/ml) or pTau− (<78 pg/ml), using accepted cutoff values.

Participants were excluded if they were diagnosed with vascular cognitive impairment or vascular dementia. Clinical diagnoses were made by neurologists and criteria included whether the patient had a known vascular brain injury, and whether 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, the 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 ischaemic 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.

Clinical exam
Participants underwent clinical assessments according to UDS procedures harmonized across all study sites, including clinical interview and review of any neurocognitive symptoms and health history with the participant and a knowledgeable informant. A general physical and neurologic exam was conducted. The CDR assessment was conducted in accordance with published standardization procedures, including standardized interview and assessment with the participant and a knowledgeable informant. In accordance with current diagnostic models for cognitive and biological research criteria for cognitive impairment and AD7, participants were separately stratified by cognitive impairment and AD biomarker abnormality using established cutoffs for CSF Aβ1−42 and pTau24,25. Cognitive impairment was determined on the basis of global CDR score and neuropsychological impairment in one or more cognitive domains.

Vascular risk factors
The vascular risk factor (VRF) burden in each participant was evaluated through physical examination, blood tests, and clinical interviews with the participant and informant; history of cardiovascular disease (heart failure, angina, stent placement, coronary artery bypass graft, intermittent claudication), hypertension, hyperlipidaemia, type 2 diabetes, atrial fibrillation, and transient ischaemic attack or minor stroke were investigated. The total VRF burden was defined by the sum of these risk factors, as previously described. We assigned an elevated VRF burden to individuals with two or more VRFs. This threshold was adopted because previous studies showed that the presence of two or more VRFs is associated with occult cerebrovascular disease at autopsy in older adults with AD, whereas a single VRF is common and not necessarily associated with increased cerebrovascular disease in this population.

Cognitive domain impairment evaluation
Impairment in one or more cognitive domain was judged by performance on comprehensive neuropsychological testing, using previously described neuropsychological criteria for cognitive impairment. All participants underwent neuropsychological testing that included the UDS Battery (version 2.0 or 3.0) plus supplementary neuropsychological tests at each site. Raw test scores were converted to age-, sex- and education-corrected z scores using the National Alzheimer’s Coordinating Center (NACC) regression-based norming procedures (https://www.alz.washington.edu/). Normalized z scores from ten neuropsychological tests were evaluated in determining domain impairment, including three tests per cognitive domain (memory, attention/executive function and language) and one test of global cognition. Impairment in one or more cognitive domains was determined using previously described neuropsychological criteria, and was defined as a score >1 s.d. below norm-referenced values on two or more tests within a single cognitive domain or three or more tests across cognitive 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. Participants were excluded from cognitive domain analyses if they had less than 90% complete neuropsychological test data (53, 24, and 82 participants were excluded for MRI, PET, and CSF analyses, respectively). Included participants were classified as 0, 1, or 2+ based on the number of cognitive domains for which they had two or more impaired test scores.
Test battery specifics for each UDS version and recruitment site are as follows. i) Global cognition: MMSE for UDS version 2 and MoCA for UDS version 3. ii) Memory: The Logical Memory Story A immediate and Delayed free recall tests (modified from the original Wechsler Memory Scales, Third Edition (WMS-III)) for UDS version 2 and the Craft Stories Immediate and Delayed free recall for UDS version 3. For supplementary tests the USC participants underwent the California Verbal Learning Test, Second Edition (CVLT-II) and the Selective Reminding Test (SRT) sum of free recall trials. Norm-referenced scores for these supplementary test scores were derived from a nationally representative sample published with the test manual (CVLT-II) and in studies of normally aging adults (SRT). iii) Attention and executive function: The Trails A, Trails B, and Wechsler Adult Intelligence Scale—Revised (WAIS-R) Digit Span Backwards tests for UDS version 2 and the Trails A, Trails B and Digit Span Backwards tests for UDS version 3. iv) Language: The Animal Fluency, Vegetable Fluency, and Boston Naming Tests for UDS version 2 and Animal Fluency, Vegetable Fluency, and Multilingual Naming Test (MINT) for UDS version 3.

Magnetic resonance imaging and analysis
The MRI data sets were obtained at the Mark and Mary Stevens Neuroimaging and Informatics Institute of USC and Washington University of St. Louis. We developed a standardized high-resolution 3T MRI brain scan protocol. At the USC site, a Siemens 3T Prisma scanner was used with a product 32-channel head receive coil and body transmit coil. At the WashU site, a Siemens 3T mMR with 20-channel head coil and Siemens 3T Vivida with 64-channel head coil were used. Anatomical coronal spin echo T2-weighted scans were first obtained through the hippocampi (TR/TE 8020/50 ms, NEX = 1, slice thickness 2 mm with 2 mm gap between slices, FOV = 175 × 175 mm, matrix size = 448 × 448). Baseline coronal T1-weighted maps were then acquired using a T1-weighted 3D volumetric interpolated breath-hold (VIBE) sequence and variable flip angle method using flip angles of 2°, 5°, 10°, 12°, and 15°. Coronal DCE-MRI covering the hippocampi and temporal lobes was acquired using a T1-weighted 3D VIBE sequence (FA = 15°, TR/TE = 5.14/2.18 ms, NEX = 1, slice thickness 5 mm with no gap, FOV 175 × 175 mm, matrix size 320 × 320, voxel size 0.550 × 0.550 × 5 mm3). This sequence was repeated for a total of 16 min with an approximate time resolution of 15.4 s. Gadolinium-based contrast agent (GBCA), 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. The standardization and optimization of the MRI protocol required several tests performed on a phantom. Specifically, scanner characterization and calibration sequences including B0, T1, and variable flip-angle mapping were implemented, optimized, and applied. After the achievement of good results in terms of quality control and reproducibility, we standardized and employed the same pre-contrast and dynamic T1-weighted protocols at both USC and Washington University sites. Of note, all the other MR sequences were also identical on both scanners.

In order to minimize inter-site variability, the entire MRI protocol including the anatomical and DCE pulse sequences were 100% mirrored from one site to another and the same contrast agent gadoterate meglumine (Dotarem, Guerbet, France) was administered intravenously in 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. The standardized and optimization of the MRI protocol required several tests performed on a phantom. Specifically, scanner characterization and calibration sequences including B0, T1, and variable flip-angle mapping were implemented, optimized, and applied. After the achievement of good results in terms of quality control and reproducibility, we standardized and employed the same pre-contrast and dynamic T1-weighted protocols at both USC and Washington University sites. Of note, all the other MR sequences were also identical on both scanners.

The consistency of the results from the two sites was additionally confirmed by our previous publication6. In brief, we performed the analysis of the combined DCE data sets from both USC and WashU sites, and additionally site-specific analysis for each of the two sites separately, which showed no statistically significant differences across sites. Recently, we invited a subset of 52 participants for an additional T1-weighted scan without contrast (using the same scanner and same MR pulse sequences) after their first DCE-MRI and measured both $B_0$ and T1 values at a two-year interval. This study showed that the results were unchanged and consistent across the scans, supporting minimal intra-site variability.

Quantification of BBB permeability
See Supplementary Information, Supplementary Methods.

Quantification of regional brain volumes
HC and PHG morphometry were performed using the FreeSurfer (v5.3.0) software package7 (http://surfer.nmr.mgh.harvard.edu/), as previously described7. The HC and PHG were segmented using FreeSurfer Desikan Killanay and subcortical atlases10,11. Then, regional volumes (mm3) were derived accordingly. The technical details of this procedure have been described previously12-14. Data processing and visualization were performed using the Laboratory of Neuro Imaging (LONI) pipeline system (http://pipeline.loni.usc.edu) and Quantitative Imaging Toolkit15-16.

Positron emission tomography and analysis
PET image acquisition was performed at the Molecular Imaging Center of USC or Mallinckrodt Institute of Radiology of WashU. Amyloid and tau PET studies were conducted using 18F-florbetaben (FBB) or 18F-flortaucipir (FBP) and 18F-flortaucipir (AV1451), respectively. FBB (Life Molecular Imaging, Inc.) was obtained from SOPHIE, Inc. for the USC site, while FBP was provided by Eli Lilly and Company for the WashU site. For all amyloid PET analysis the FBP and FBB data sets were combined. AV1451 was provided by Avid Radiopharmaceuticals, Inc. for the USC site and was produced by the Mallinckrodt Institute of Radiology for the WashU site. A Siemens Biograph 64 PET scanner was used at the USC site. At the WashU site, FBP scans were acquired on a Siemens mMR and AV1451 scans were acquired on a Siemens Biograph mCT.

In brief, a computed tomography (CT) scan was performed first for attenuation correction before each PET imaging session. The downloaded PET images from FBB, FBP, and AV1451 tracers were processed by using standard uptake value maps (SUV in g/ml). All PET images were co-registered to structural high-resolution 3D T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MP-RAGE) MRI images using FSL-FLIRT (FMRIB's Linear Image Registration Tool)10. The FreeSurfer-segmented cerebellum was used as a reference tissue to normalize for both amyloid and tau17.

After co-registration of PET images into an anatomical reference image (MNI152 standard-space), Statistical Parametric Mapping (SPM12) was used for group comparison on a voxel-by-voxel basis. Age at time of PET imaging session, sex, and education were introduced in a multiple regression model as covariates. Level of significance was set to $P < 0.001$ for amyloid and $P < 0.005$ for tau (uncorrected Pvalues) with the minimum number of voxels (Ke) in a cluster of 50.

Additionally, given the known AV1451 off-target ligand binding in the choroid plexus (CP)18,19, which can contribute to HC regional AV1451 signal owing to the close proximity of the CP to the HC and relatively low spatial resolution of PET scans (that is, ~6-mm voxel size), we took advantage of visualizing CP by DCE-MRI, also performed in these individuals, which allowed us to subtract the contribution of the CP signal to the HC AV1451 proper signal. The following steps were used to correct
for off-target ligand binding to the CP (see Extended Data Fig. 5). Step I: HC masks were generated from the 3D T1-weighted MP-RAGE. Step 2: CP masks were generated from the T1-weighted VIBE post-GBCA (FA = 15°) image. Step 3: HC and CP masks were overlaid. Step 4: The overlap of the CP and HC masks was subtracted to obtain a CP-corrected HC PET signal after adding 6-mm voxel size on top of the CP mask generated from DCE data. Representative images of HC AV1451 PET signal before and after applying the CP correction are shown in Extended Data Fig. 5.

We next quantified regional changes in amyloid and tau SUV ratio (SUVR) in relation to regional DCE-MRI Ktrans values in all participants stratified by APOE genotype. The regional SUVR values were taken from the FreeSurfer-segmented HC, PHG, OFC28, and ITG29. The BBB Ktrans constant (DCE-MRI) was determined in all participants (Extended Data Tables 2a, b). This includes those who were analysed for both amyloid and tau (n = 38), only amyloid (n = 9) or only tau (n = 29).

**Lumbar puncture and venipuncture**
Participants underwent a lumbar puncture and venipuncture in the morning after an overnight fast. The CSF was collected in polypropylene tubes, processed (centrifuged at 2,000g, 4 °C, 10 min USC site; 5 min WashU site), aliquoted into polypropylene tubes and stored at −80 °C until assay. Blood was collected into EDTA (EDTA) tubes and processed (centrifuged at 2,000g, 4 °C, 10 min USC site; 5 min WashU site). 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-gDNA Blood Miniprep Kit (catalogue no. D3024, Zymo Research, Irvine, CA). APOE genotyping was performed via polymerase chain reaction (PCR)-based retention fragment length polymorphism analysis, as previously reported.

**Molecular assays**

**Quantitative western blotting of sPDGFRβ.** The quantitative western blot analysis was used to detect sPDGFRβ in human CSF (ng/ml), as previously reported.

**BBB breakdown biomarkers.** Albumin quotient (Qalbumin), the ratio of CSF to plasma albumin levels) and CSF levels of fibrinogen and plasminogen were determined using enzyme-linked immunosorbent assay (ELISA), as previously reported.

**Cyclophilin A.** We developed a novel CypA assay on the Meso Scale Discovery (MSD) platform. Standard-bind 96-well plates (catalogue no. L15XXA-3/L11XXA-3, MSD, Rockville, MD) were spot-coated with 5 µl per well of 40 µg/ml rabbit polyclonal anti-Cyclophilin A antibody (catalogue no. 10436-T52, Sino Biological, Wayne, PA) prepared in 0.03% Triton X-100 in 0.01 M PBS pH 7.4 solution. The plates were left undisturbed overnight to dry at room temperature. The next day, the plates were blocked with 150 µl per well of Blocking One (catalogue no. 03953-95, Nacalai Tesque, Japan) and incubated for exactly 1 h with shaking. Meanwhile, samples and standards were prepared in Blocking One blocking buffer. Different concentrations ranging from 3.5 to 200 ng/ml of a recombinant human Cyclophilin A protein (catalogue no. 3589-CAB, R&D Systems, Minneapolis, MN) were used to generate a standard curve. All CSF samples were diluted 1:3. After blocking, the plates were manually washed three times with 200 µl per well of wash buffer (in 0.05% Tween-20 in 0.01 M PBS pH 7.4). The prepared samples or standards were added at 25 µl per well, and the plates were incubated overnight at 4 °C with shaking. The next day, the plates were washed three times, and 25 µl per well of 1 µg/ml sulfo-tagged mouse monoclonal Cyclophilin A detection antibody (catalogue no. ab13144, Abcam, Cambridge, MA), prepared in Blocking One. The plates were incubated for 90 min at room temperature with shaking. Next, the plates were washed four times, then 150 µl per well of 2× Read Buffer T with surfactant (catalogue no. R92TC-3, MSD, Rockville, MD) was added and the plates were read immediately on an MSD SECTOR Imager 6000 (MSD, Rockville, MD) with electrochemiluminescence detection.

The raw readings were analysed by subtracting the average background value of the zero standard from each recombinant standard and sample reading. A standard curve was constructed by plotting the recombinant standard readings and their known concentrations and applying a nonlinear four-parameter logistics curve fit. The Cyclophilin A concentrations were calculated using the samples’ reading and the standard curve equation; the result was corrected for the sample dilution factor to arrive at the Cyclophilin A concentration in the CSF samples.

**Matrix metalloproteinase-9.** CSF levels of MMP9 were determined using the human MMP9 Ultra-Sensitive Kit from MSD (cat. No. K151HAC).

**Neuron-specific enolase.** CSF levels of NE were determined using ELISA (cat. No. E-80NEN, Immunology Consultant Laboratories, Portland, OR). The company no longer sells this product; thus, this analyte was measured in the majority of participants but not in those individuals that enrolled in the study most recently.

**S100B.** CSF levels of the astrocyte-derived cytokine S100 calcium-binding protein B (S100B), were determined using ELISA (cat. no. EHZS100B-33K, EMD Millipore, Billerica, MA).

**Inflammatory markers.** An MSD multiplex assay was used to determine CSF levels of intercellular adhesion molecule 1 (ICAM1) (cat. no. K15198D, MSD, Rockville, MD), and interleukin-6 (IL6), II-β, tumor necrosis factor-α (TNFα), and interferon gamma (IFNγ) (cat. no. K15049G, MSD, Rockville, MD).

**Aβ peptides.** An MSD multiplex assay (cat. no. K15200E, MSD, Rockville, MD) was used to determine CSF levels of Aβ42, Aβ40. Participants were stratified based on CSF analysis as either Aβ+ (>190 pg/ml) or Aβ− (<190 pg/ml) using the accepted cutoff values as previously reported for the MSD 6E10 Aβ peptide assay.

**Tau.** Phosphorylated tau (pT181) was determined by ELISA (cat. no. S16181, Innotest, Fujirebio US, Inc., Malvern, PA). Participants were stratified based on CSF analysis as either pTau+ (>78 pg/ml) or pTau− (<78 pg/ml), using the accepted cutoff value as previously reported.

**Human iPSCs**

iPSC lines were generated by reprogramming of skin fibroblasts from APOE ε4/ε4 and APOE ε3/ε3 donors with AD or without (control) as recently reported. Reprogramming was performed using integration-free Sendai virus vectors and we passaged cells to passage 15 and confirmed normal karyotype. hiPSCs were maintained on Matrigel (Corning) in mTeSR1 (catalogue no. 85850, StemCell Technologies, Vancouver, BC, Canada) supplemented with 10 ng/ml FGF2 StemBeads (StemCultures) or mTeSR plus (StemCell Technologies) every other day.

**Differentiation of iPSCs into pericytes**

Differentiation of iPSCs into pericytes was carried out as described previously. In brief, iPSCs were dissociated with ReLeSR (catalogue no. 05872, StemCell Technologies) and seeded at 55,000 cells/cm² in Essential 8 medium (catalogue no. A1517001, ThermoFisher, Waltham, MA, USA) supplemented with ROCK inhibitor Y-27632 (10 µM, catalogue no. 72304, StemCell Technologies) on Matrigel (0.5 mg/6-well plate, catalogue no. 354230, Corning, NY, USA). After 24 h incubation, the iPSCs were switched into STEMdiff Mesoderm Induction Medium (MIM, catalogue no. 05221, StemCell Technologies) for 5 days with daily medium change. On day
6 of MIM treatment, the cells were plated on Matrigel at 25,000 cells/cm² in pericyte medium (catalogue no. 1201, ScienCell, Carlsbad, CA, USA) for an additional 7 days. The differentiated cells were dissociated with Accutase (catalogue no. 07920, StemCell Technologies). Following incubation with human PDGFRβ biotinylated antibody (catalogue no. BAF385, R&D Systems, Minneapolis, MN, USA), the cells were incubated with anti-biotin microbeads (catalogue no. L30-090-485, Miltenyi Biotec, Bergisch Gladbach, NRW, Germany) and magnetically sorted using MACS LS columns (catalogue no. L30-042-401, Miltenyi Biotec) following the manufacturer’s instructions. Sorted pericytes were plated at a density of 25,000 cells/cm² on Matrigel-coated coverslips for immunocytochemistry analyses or poly-l-lysine-coated six-well culture plates for western blot analyses. Differentiated pericytes were positive for the pericyte markers PDGFRβ, CD13, and NG2, and negative for the endothelial marker CD31, the astrocytic marker GFAP, and the microglial marker CD11b.

**Statistical analyses**

Prior to performing statistical analyses, we first screened for outliers using the Grubbs’ test, also called the ESD (extreme studentized deviate) method, applying a significance level of α = 0.01 (https://www.graphpad.com/quickcalcs/grubbs1/). For each of the outliers identified, a secondary index of outlier influence was applied using the degree of deviation from the mean (greater than ± 3.5 σ). Using these stringent criteria, a total of five outliers (one each in Figs. 1, j and k) and two each in Extended Data Fig. 6a, b, were removed from analyses, as indicated in the legends of these figures. Continuous variables were also evaluated for departures from normality through quantitative examination of skewness and kurtosis, in addition to visual inspection of frequency distributions. Where departures of normality were identified, log10 transformations were applied, and distribution normalization was confirmed before parametric analyses. This was done for Fig. 4h, k and Extended Data Fig. 7a, b, d, e. As the use of log10 transformations accounts for any non-normality, this obviated the need for outliers exclusion.

**DCE-MRI $K_{trans}$ and CSF sPDGFRβ and CypA.** Regional DCE-MRI $K_{trans}$ values and CSF sPDGFRβ, CypA and MMP9 levels were compared across the entire sample stratified by APOE status. As in the APOE4 group relatively few participants were homozygous ε4/ε4 compared to heterozygous ε3/ε4 (14% for DCE-MRI analysis, and 18% for sPDGFRβ analysis), and initial comparisons between ε3/ε4 and ε3/ε4 carriers did not show any significant differences in regional HC and PHG DCE-MRI $K_{trans}$ values (CDR 0, $P_{HC} = 0.19$ and $P_{PHG} = 0.54$ (PHG); CDR 0.5, $P_{HC} = 0.22$ and $P_{PHG} = 0.84$) or CSF sPDGFRβ levels (CDR 0, $P = 0.23$; CDR 0.5, $P = 0.47$), all subsequent analyses combined APOE4 carriers (ε3/ε4 and ε4/ε4), and compared these participants to APOE3 carriers (ε3/ε3) stratified by cognitive impairment status (CDR 0 versus 0.5 and 0 versus 1 versus 2 cognitive domain impairment using ANCOVA with FDR correction for multiple comparisons (see details below)). For DCR analyses, model covariates included age, sex, and education. Cognitive domain impairment was determined using age-, sex-, and education-corrected values, so these covariates were not additionally included in the analyses. Additional post hoc ANCOVA analyses evaluated whether the observed differences remained significant after stratifying APOE4 carriers by CSF $\text{A}_\beta_{\text{42}}$ and pTau status, and after statistically controlling for CSF $\text{A}_\beta_{\text{42}}$ and pTau status and regional brain volume in APOE4 non-carriers and carriers. These findings were also confirmed by hierarchical logistic regression models using the same covariates.

**PET AD biomarkers.** In a subset of participants who underwent amyloid and tau PET imaging together with DCE-MRI studies, we used ANCOVA models controlled for age, sex and education to compare regional amyloid and tau ligand binding and DCE-MRI values in a set of APOE4 non-carriers and carriers within a priori regions of interest, based on prior imaging studies, to determine whether distinct regional pathologies differed by APOE4 carrier status.

**Baseline CSF sPDGFRβ as a continuous predictor of cognitive decline.** For linear mixed model analysis, baseline CSF sPDGFRβ was a continuous predictor of demographically corrected global cognitive change at 2-year follow up intervals, controlling for CSF $\text{A}_\beta_{\text{42}}$ and CSF pTau status. Global cognition was indexed by age-, sex-, and education-corrected z scores on mental status exam (MMSE or MoCA) and as the global cognitive composite of all age-, sex-, and education-corrected neuropsychological test z scores (see above for list of neuropsychological tests). Time was modelled with date of LP as baseline (t0) with two follow-up intervals of 2 years each (t1, t2). Additional analyses confirmed all findings when time was modelled as time since baseline, with date of lumbar puncture as baseline (t0) and follow up as annual intervals (t1–t n).

All longitudinal mixed models treated CSF sPDGFRβ as a continuous predictor. Although we have previously established that CSF sPDGFRβ is a marker of pericyte injury, the optimal cutoff value for abnormal CSF sPDGFRβ levels indicative of pericyte injury remains unknown.

**Correlational analyses.** Pearson product moment correlations were used to evaluate relationships among CSF sPDGFRβ, CypA, MMP9, fibrinogen, plasminogen and hippocampal and parahippocampal BBB $K_{trans}$ levels among APOE4 carriers.

**Multiple comparison correction and missing data.** Given the large number of analyses, FDR correction was applied to $P$ values for primary study outcomes (DCE-MRI, sPDGFRβ) evaluated in the entire sample by APOE4 carrier status and CDR status using the Benjamini–Hochberg method in ANCOVA and logistic regression models controlling for age, sex, education, brain volume, and CSF $\text{A}_\beta_{\text{42}}$ and pTau status (for DCE-MRI analyses). Post hoc confirmatory analyses in participant subsets further evaluating independence of CSF and PET markers of amyloid and tau, evaluation of mechanistic markers (that is, CypA and MMP9), and longitudinal analysis of predictive value of CSF sPDGFRβ were not corrected for multiple comparisons. For longitudinal data with variable follow up, we used linear mixed model analyses with and accounted for missing data via the missing at random assumption.

**Reporting summary**

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

**Data availability**

All data generated and/or analysed during this study are either included in this article (and its Supplementary Information) or are available from the corresponding author on reasonable request. Source Data for Figs. 1–4 are provided with the article.

**Code availability**

All software used in this study are publicly available: Rocketship v1.2 (https://github.com/petmri/ROCKETSHIP/blob/master/dce-compare_gui.m), FreeSurfer (v5.3.0) (http://surfer.nmr.mgh.harvard.edu/),...
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Author contributions A.M., D.A.N., A.P.S., G.B., M.D.S. and B.V.Z. designed the research study and analysed and interpreted the data. A.M., D.A.N., A.P.S., G.B., M.D.S., A.C., M.F. and Y.C. performed the experiments and analysed the data. A.M. and G.B. performed the MRI analysis. A.M., G.B. and A.C. performed the PET analysis. A.P.S., M.D.S. and M.P. performed the biofluids analysis. D.A.N. performed the neurophysiological analysis. A.P.S., Y.C., B.V.Z. and J.TCW. contributed to human iPSC-pericyte experiments. L.M.D. and A.R.N. prepared and submitted the study to the IRB. M.P., E.J., D.P.B., M.G.H., T.L.S.B., A.M.F., J.M.R., L.S.S., J.C.M., E.M.R., R.J.C., H.C.C., J.TCW, J.P., P.S.C., M.L. and A.W.T. recruited the participants and performed and provided the imaging scans. A.P.S., G.B., M.G.H., T.L.S.B., A.M.F., J.M.R., L.S.S., J.C.M., E.M.R., R.J.C., H.C.C., J.TCW, P.S.C. and A.W.T. provided critical reading of the manuscript. A.M. and D.A.N. contributed to manuscript writing and B.V.Z. wrote the manuscript.

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FSL-FLIRT (https://fsl.fmrib.ox.ac.uk/fslwiki/FLIRT), SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/), and Quantitative Imaging Toolkit (https://cabeen.io/about/publication/cabeen2018quantitative/).
Extended Data Fig. 1 | Regional BBB $K_{\text{trans}}$ constant in eight additional brain regions in APOE4 carriers and non-carriers (APOE3) with CDR status 0 and 0.5. BBB $K_{\text{trans}}$, constant in the ITG (a), superior frontal gyrus (SFG, b), caudate nucleus (CN, c), thalamus (Thal, d), striatum (Str, e), subcortical watershed normal-appearing white matter (Subcort. WS NAWM, f), corpus callosum (CC, g), and internal capsule (IC, h) in individuals with CDR 0 bearing APOE3 (black, $n = 128$) and APOE4 (red, $n = 68$), and with CDR 0.5 bearing APOE3 (black, $n = 14$) and APOE4 (red, $n = 25$). Continuous lines, median; dotted lines, IQR. Significance by ANCOVAs for main effects and post hoc comparisons controlling for age, sex, and education.
**Extended Data Fig. 2 | BBB breakdown in the HC and PHG in APOE4 carriers increases with cognitive domain impairment.**

*a, b*, $K_{trans}$ constant in the HC (a) and PHG (b) in individuals with no cognitive domains impaired bearing APOE3 (black, $n = 70$) or APOE4 (red, $n = 40$); one cognitive domain impaired bearing APOE3 ($n = 18$) or APOE4 ($n = 21$); and two or more cognitive domains impaired bearing APOE3 ($n = 7$) or APOE4 ($n = 12$). Continuous lines, median; dotted lines, IQR.  

*c, d*, $K_{trans}$ (estimated marginal mean ± s.e.m. from ANCOVA models corrected for age, sex, education, CSF Aβ 1–42 and pTau status, and HC and PHG volumes) in the HC (c) and PHG (d) in individuals with no cognitive domains impaired bearing APOE3 ($n = 70$) or APOE4 ($n = 40$); one cognitive domain impaired bearing APOE3 ($n = 18$) or APOE4 ($n = 21$); and two or more cognitive domains impaired bearing APOE3 ($n = 7$) or APOE4 ($n = 12$). Significance by ANCOVA for main effects and post hoc comparisons controlling for age, sex, and education. All ANCOVA omnibus tests remained significant at FDR threshold of 0.05.
Extended Data Fig. 3 | Regional BBB $K_{\text{trans}}$ constant in eight additional brain regions in APOE4 carriers and APOE3 carriers with different degrees of cognitive domain impairment. $K_{\text{trans}}$ constant in the ITG (a), SFG (b), CN (c), thalamus (d), striatum (e), subcortical WS NAWM (f), CC (g), and IC (h) in individuals with no cognitive domains impaired bearing APOE3 (black, $n=70$) or APOE4 (red, $n=40$); one cognitive domain impaired bearing APOE3 ($n=18$) or APOE4 ($n=21$); and two or more cognitive domains impaired bearing APOE3 ($n=7$) or APOE4 ($n=12$). Continuous lines, median; dotted lines, IQR. Significance tests from ANCOVAs for main effects and post hoc comparisons controlling for age, sex, and education.
Extended Data Fig. 4 | Regional BBB $K_{\text{trans}}$ constant in all studied brain regions in APOE4 carriers and APOE3 carriers in relation to vascular risk factors. $K_{\text{trans}}$ constant in the HC (a), PHG (b), ITG (c), SFG (d), CN (e), thalamus (f), striatum (g), subcortical WS NAWM (h), CC (i), and IC (j) in APOE3 (green, $n = 80$) and APOE4 (brown, $n = 42$) carriers with 0–1 vascular risk factors (VRFs), and APOE3 ($n = 58$) and APOE4 ($n = 51$) carriers with 2+ VRFs. Continuous lines, medians; dotted lines, IQR. Significance by ANCOVAs for main effects and post hoc comparisons controlling for age, sex, and education.
Extended Data Fig. 5 | Amyloid and tau PET analysis in APOE4 carriers and correction of 18F-AV1451 off-target binding in the choroid plexus. All studies were performed in individuals with CDR score 0. Amyloid and tau PET studies were conducted using 18F-FB or 18F-FBP, and 18F-AV1451, respectively. For amyloid PET data analysis, FBP and FBB data sets were combined. a, Uptake of amyloid tracers by the OFC in APOE4 (n = 29) relative to APOE3 (n = 45) carriers (voxel-wise two-sample one-tailed t-tests). b, Representative amyloid PET SUVR maps from an APOE3 homozygote (top) and an APOE4 carrier (APOE4) (bottom). Slices 1 and 2, regions of interest (ROIs) for amyloid PET and BBB DCE-MRI scans (see e). Arrow, amyloid tracer uptake by OFC. The APOE3 and APOE4 representative images used FBP. c, Uptake of tau tracer shows undetectable tau accumulation in APOE3 (n = 60) or APOE4 (n = 37) carriers (voxel-wise two-sample one-tailed t-tests). d, Representative tau PET SUVR maps from APOE3 (top) and APOE4 (bottom) carriers. Slices 1 and 1’, ROIs for tau PET and BBB DCE-MRI scans, respectively (see e). e, Coronal 3D scans of regions studied in Fig. 2: HC (red), PHG (green), medial OFC (yellow), and ITG (blue). f, Correction of 18F-AV1451 off-target binding in the choroid plexus. Step 1, HC masks were generated from the 3D T1-weighted magnetization prepared–rapid gradient echo (MP-RAGE). Step 2, CP masks were generated from the T1-weighted VIBE post-GBCA image (flip angle, 15°). Step 3, HC and CP masks were overlaid (arrowheads, red). Step 4, areas of CP overlap with HC masks (arrowheads, yellow) were subtracted to obtain CP-corrected HC tau PET signal after adding 6-mm voxel size on top of CP mask generated from DCE data. g, Representative images of HC tau PET signal before (top) and after (bottom) applying the CP correction (arrows and white dotted lines show overlap between HC and CP).
Extended Data Fig. 6 | CSF biomarkers of glia and inflammatory response and endothelial and neuronal cell injury in APOE4 and APOE3 carriers.

a, CSF astrocytic S100B levels in individuals with CDR 0 bearing APOE3 (black, n = 77) or APOE4 (red, n = 41), and with CDR 0.5 bearing APOE3 (n = 39) or APOE4 (n = 32). b, CSF IL6 levels in individuals with CDR 0 bearing APOE3 (n = 71) or APOE4 (n = 47), and with CDR 0.5 bearing APOE3 (n = 34) or APOE4 (n = 32). c, CSF IFNγ levels in individuals with CDR 0 bearing APOE3 (n = 54) or APOE4 (n = 29), and with CDR 0.5 bearing APOE3 (n = 25) or APOE4 (n = 17). d, CSF IL1β levels in individuals with CDR 0 bearing APOE3 (n = 43) or APOE4 (n = 18), and with CDR 0.5 bearing APOE3 (n = 17) or APOE4 (n = 13). e, CSF TNFα levels in individuals with CDR 0 bearing APOE3 (n = 70) or APOE4 (n = 46), and with CDR 0.5 bearing APOE3 (n = 34) or APOE4 (n = 32). f, CSF soluble intercellular adhesion molecule 1 (sICAM1) levels in individuals with CDR 0 bearing APOE3 (n = 77) or APOE4 (n = 40), and with CDR 0.5 bearing APOE3 (n = 39) or APOE4 (n = 33). g, CSF NSE levels in individuals with CDR 0 bearing APOE3 (n = 47) or APOE4 (n = 32), and with CDR 0.5 bearing APOE3 (n = 29) or APOE4 (n = 29). Continuous lines, median; dotted lines, IQR. a and b had one outlier each, which were removed before statistical analysis (see Methods). Significance by ANCOVAs for main effects and post hoc comparisons controlling for age, sex, and education.
Extended Data Fig. 7 | Decreased CSF Aβ1-42 and increased pTau levels in APOE4 carriers with cognitive impairment. 

**a**, CSF Aβ1-42 levels in individuals with CDR 0 bearing APOE3 (black, n = 141) or APOE4 (red, n = 83) and with CDR 0.5 bearing APOE3 (n = 39) or APOE4 (n = 41). **b**, CSF Aβ1-42 levels in APOE3 (n = 89) and APOE4 (n = 55) carriers with no cognitive domains impaired, APOE3 (n = 29) and APOE4 (n = 31) carriers with one cognitive domain impaired, and APOE3 (n = 17) and APOE4 (n = 14) carriers with two or more cognitive domains impaired. **c**, CSF Aβ1-42 levels (estimated marginal means ± s.e.m. from ANCOVA models corrected for age, sex, and education, and CSF sPDGFRβ levels) in individuals with CDR 0 bearing APOE3 (n = 141) or APOE4 (n = 83) and with CDR 0.5 bearing APOE3 (n = 39) or APOE4 (n = 41). **d**, CSF pTau levels in individuals with CDR 0 bearing APOE3 (n = 141) or APOE4 (n = 82) and with CDR 0.5 bearing APOE3 (n = 39) or APOE4 (n = 43). **e**, CSF pTau levels in APOE3 (n = 89) and APOE4 (n = 56) carriers with no cognitive domains impaired, APOE3 (n = 29) and APOE4 (n = 30) carriers with one cognitive domain impaired, and APOE3 (n = 17) and APOE4 (n = 15) carriers with two or more cognitive domains impaired. **f**, CSF pTau levels (estimated marginal means ± s.e.m. from ANCOVA models corrected for age, sex, education, and CSF sPDGFRβ levels) in individuals with CDR 0 bearing APOE3 (n = 141) or APOE4 (red, n = 82) and with CDR 0.5 bearing APOE3 (n = 39) or APOE4 (n = 43). Violin plots: continuous lines, median; dotted lines, IQR. CSF Aβ1-42 and pTau values were log10-transformed before statistical analysis because they had a non-normal distribution. Significance tests from ANCOVAs for main effects and post hoc comparisons controlling for age, sex, and education.
Extended Data Fig. 8 | Full scans of western blots. Full scans of western blots for CypA shown in Fig. 4m (top).
### Extended Data Table 1 | APOE3 and APOE4 carriers studied for regional BBB permeability changes by DCE-MRI

| APOE Genotype                      | APOE3 | APOE4 | APOE3 | APOE4 |
|-----------------------------------|-------|-------|-------|-------|
| Clinical Dementia Rating (CDR)    | 0     | 0     | 0.5   | 0.5   |
| No. of participants               | 130   | 76    | 14    | 25    |
| Age at MRI, years, Mean (SD)      | 69.9 (7.9) | 67.3 (8.7) | 73.8 (8.3) | 69.4 (8.7) |
| Female, %                         | 62.3  | 57.9  | 42.9  | 56    |
| Education, years, Mean (SD)       | 16.6 (2.7) | 16.7 (2.0) | 16.4 (2.5) | 17.1 (2.1) |
| Cognitive domain impairment, No. 0, 1, 2+ | 78, 17, 2 | 38, 16, 4 | 6, 2, 6 | 6, 8, 9 |
| Vascular risk factors, No. 0-1, 2+ | 77, 53 | 38, 38 | 8, 6  | 10, 15 |
**Extended Data Table 2** | APOE3 and APOE4 carriers studied for regional amyloid or tau brain accumulation by PET and BBB permeability changes by DCE-MRI

| APOE Genotype | APOE3 | APOE4 |
|---------------|-------|-------|
| **Clinical Dementia Rating (CDR)** | 0     | 0     |
| **No. of participants** | 45    | 29    |
| **No. of participants (FBB, FBP)** | 5, 40 | 9, 20 |
| **Age at amyloid PET, years, Mean (SD)** | 68.4 (7.5) | 65.7 (8.8) |
| **Female, %** | 73.3  | 65.5  |
| **Education, years, Mean (SD)** | 16.7 (2.7) | 16.5 (2.1) |
| **Cognitive domain impairment, No. 0, 1, 2+** | 24, 4, 0 | 17, 4, 1 |
| **Vascular risk factors, No. 0-1, 2+** | 23, 22 | 19, 10 |

| APOE Genotype | APOE3 | APOE4 |
|---------------|-------|-------|
| **Clinical Dementia Rating (CDR)** | 0     | 0     |
| **No. of participants** | 60    | 37    |
| **Age at tau PET, years, Mean (SD)** | 68.7 (7.9) | 64.0 (8.4) |
| **Female, %** | 66.6  | 37.8  |
| **Education, years, Mean (SD)** | 16.5 (2.7) | 16.6 (2.1) |
| **Cognitive domain impairment, No. 0, 1, 2+** | 27, 5, 1 | 15, 6, 1 |
| **Vascular risk factors, No. 0-1, 2+** | 36, 24 | 23, 14 |

*a, Participants studied for amyloid brain accumulation; b, participants studied for tau accumulation. FBB, participants who received 18F-Florbetaben; FBP, participants who received 18F-Florbetapir.*
## Extended Data Table 3 | APOE3 and APOE4 carriers studied for CSF sPDGFRβ levels

| APOE Genotype                  | APOE3 | APOE4 | APOE3 | APOE4 |
|-------------------------------|-------|-------|-------|-------|
| Clinical Dementia Rating (CDR)| 0     | 0     | 0.5   | 0.5   |
| No. of participants           | 157   | 105   | 40    | 48    |
| Age at LP, years, Mean (SD)   | 70.2 (8.9) | 67.3 (9.9) | 76.5 (7.3) | 72.8 (8.1) |
| Female, %                     | 64    | 61    | 39    | 37    |
| Education, years, Mean (SD)   | 16.5 (2.7) | 16.3 (2.3) | 15.7 (2.8) | 16.2 (2.8) |
| Cognitive domain impairment, No. 0, 1, 2+ | 91, 20, 2 | 62, 17, 2 | 9, 8, 14 | 12, 16, 15 |
| Vascular risk factors, No. 0-1, 2+ | 100, 57 | 57, 48 | 22, 18 | 18, 30 |
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

The data collection software used were SYNGO (console software) and VE11C (MRI software) for MRI, and PETSyngo 6.7.3 for PET.

Data analysis

Our Rocketship GUI code v1.2 (https://github.com/petmri/ROCKETSHIP/blob/master/dce/compare_gui.m) running with Matlab R2019a was used for DCE-MRI analyses. We also used FreeSurfer (v5.3.0) software package (http://surfer.nmr.mgh.harvard.edu/) for regional brain volume analyses. We used FSL-FLIRT (FMRIB's Linear Image Registration Tool) for PET-MR co-registration (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT), and Statistical Parametric Mapping (SPM12) was used for PET group comparison in a voxel-by-voxel basis (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Quantitative Imaging Toolkit was used for data visualization (https://cabeen.io/about/publication/cabeen2018quantitative/). Statistical analyses were conducted with a commercial statistical software package - SPSS (IBM) v26.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

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. See Data Availability statement pasted here: "All data generated and/or analyzed during this study are either included in this article (and its Supplementary Information) or are available from the corresponding author on reasonable request. Source Data for Figs. 1-4 are provided with the article".

n/a Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐ The statistical test(s) used AND whether they are one- or two-sided
☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.
☐ A description of all covariates tested
☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  Give P values as exact values whenever suitable.
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Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size

Based on prior studies demonstrating very large effect sizes for blood-brain barrier markers relating to cognitive impairment (see Montagne et al., Neuron 2015 and Nation et al., Nat Med 2019), we anticipated needing a minimum sample size of 30-20 participants per subgroup (APOE4 status x CDR status) indicating a minimum total sample size of 40-80 to be sufficient to detect significant blood-brain barrier marker effects on cognitive status in all participant subgroups. Consistent with these prior studies, very large effect sizes were observed for all primary study outcomes (up to r2 > .32). Based on these observations, a post-hoc power analysis for uncorrected comparisons indicated >99% power to detect significant differences, noncentrality parameter, delta = 5.99, critical t = 1.99, for an α = .05. In another post-hoc power analysis for corrected comparisons (ANOVA with 5 covariates), we again found >99% power to detect significant differences (noncentrality parameter, λ = 45.57, critical F = 1.9, for an α = .05). Post-hoc power analyses were conducted using a general power analysis software program (i.e., GPower).

Data exclusions

Prior to performing statistical analyses, we first screened for outliers using the Grubb's test, also called the ESD method (Extreme Studentized Deviate), applying a significance level of α=0.01 (https://www.graphpad.com/quickcalcs/grubbs1/). For each of the outliers identified, a secondary index of outlier influence was applied using the degree of deviation from the mean (greater than +/- 3 SDs). Using these stringent criteria, a total of 5 outliers each in main Figure panels 1J, 1K, and 2J, and one each in Extended Data Figs. 6a and 6b, were removed from analyses, as indicated in the legends of these figures. Continuous variables were also evaluated for departures from normality through quantitative examination of skewness and kurtosis, in addition to visual inspection of frequency distributions. Where departures of normality were identified, log10-transformations were applied, and distribution normalization was confirmed prior to parametric analyses. This has been done for main Figs. 4h and 4k, and Extended Data Figs. 7a, 7b, 7d, and 7e. Since use of log10-transformations accounts for any non-normality this obviated the need for outliers exclusion.

Further, as stated in our "Participant Inclusion and Exclusion criteria" (see Methods), we included participants (≥45 years of age) with neuropsychologically-confirmed normal cognition or mild 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 by APOE genotype as APOE4 carriers (e3/e4 and e4/e4) and APOE4 non-carriers (e3/e3) also defined as APOE3 homozygotes who were cognitively normal or mild cognitive dysfunction, as determined by clinical dementia rating (CDR) scores and the presence of cognitive impairment in one or more cognitive domains based on comprehensive neuropsychological evaluation including performance on ten neuropsychological tests assessing memory, attention/executive function, language and global cognition. For all analyses individuals with e3/e4 and e4/e4 alleles were pooled together in a single APOE4 group, as we did not find in the present cohort consisting of 82-86% e3/e4 and 14-18% e4/e4 participants (depending on the outcome measure) a significant difference between the two versus one e4 allele for the studied parameters including the BBB permeability unidirectional transfer constant ktrans values and sPDGFβR CSF values (see statistical section below). Individuals have been additionally stratified by Aβ and pTau CSF analysis as either Aβ-positive (Aβ1-42, <190 pg/mL) or Aβ-negative (Aβ1-42, >190 pg/mL), or pTau-positive (pTau+, >78 pg/mL), or pTau-negative (pTau-, <78 pg/mL), using accepted cutoff values. Participants were excluded if they were diagnosed with vascular cognitive impairment or vascular dementia. Clinical diagnoses were made by neurologists and criteria included 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

Here, we recruited the participants from two sites, University of Southern California (USC), Los Angeles, CA, and Washington University (WashU), St. Louis, MO. At the USC site, participants were recruited through the USC Alzheimer’s Disease Research Center (ADRC); combined USC and the Huntington Medical Research Institutes (HMRI), Pasadena, CA. Our previous study findings regarding CSF biomarker analyses were replicated in two independent samples (Nation et al., Nat Med 2019).

The DCE-MRI findings were also conducted at two sites (USC and WashU) using high-resolution 3T MR imaging brain scan protocol. For USC site, a Siemens 3T Prisma scanner was used with a product 32-channel head receive coil and body transmit coil. For WashU site, a Siemens 3T mMR with 2D-channel head coil and Siemens 3T Viola with 64-channel head coil were used. The standardization and optimization of the MRI protocol required several tests performed on a phantom. Specifically, scanner characterization and calibration sequences including B0, T1, and variable flip-angle mapping were implemented, optimized, and applied. After the achievement of good results in terms of quality control and reproducibility, we standardized and employed the same pre-contrast and dynamic T1-weighted protocols at both USC and Washington University sites. Of note, all the other MR sequences were identical too on both scanners. In order to minimize the inter-site variability, the entire MRI protocol including the anatomical and DCE pulse sequences were 100% mirrored from one site to another. To minimize inter-site variability, the same contrast-agent Gadoterate meglumine (Dotarem®) were injected to participants at the same concentration (0.05 mmol/kg). Finally, the same exact pre- and post-processing analysis pipeline was applied for both sites which includes T1 multi-FA mapping using linear fitting and Patlak-based DCE modeling using the arterial input function determined in each individual from the internal carotid artery. Applying all the above cited factors significantly limited inter-site variability. The consistency of the results from the two sites has been additionally confirmed by our previous publication. In brief, we performed the analysis of the combined DCE datasets from both USC and WashU sites, and additionally site-specific analysis for each of the two sites separately, which showed no statistically significant differences.
across sites. Recently, we invited a subset of 52 participants for an additional T1-weighted scan without contrast (using the same scanner and same MR pulse sequences) after their first DCE-MRI and measured both B0 and T1 values at 2-year interval. This study showed that the results were unchanged and consistent across the scans, supporting minimal intra-site variability. USC and WashU DCE-MRI datasets were acquired and processed the same way, and then combined.

The PET scans were also performed at two sites (USC and WashU). At USC site, a Siemens Biograph 64 PET scanner was used. At WashU site, FBP scans were acquired on a Siemens mMR and AV1451 scans were acquired on a Siemens Biograph mCT. The mCT session was used for attenuation correction of the mMR scans. Participants were injected with 300 MBq (±10%) of FBB or 370 MBq (±10%) of FBIP. FBB and FBIP images were acquired from 90 to 110 min and 50 to 70 min, respectively, after injection in accordance with the manufacturers’ recommendation. USC and WashU PET datasets were acquired and processed the same way, and then combined as described in the Methods.

All attempts at replication for CSF, MRI, and PET analyses across sites were successful.

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

**Blinding**
All CSF assays, DCE-MRI and PET scans were conducted by investigators who were blinded to participant demographic information, clinical diagnostic status, and genotype.

**Reporting for specific materials, systems and methods**

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☑ | ☑ |
| Antibodies | Involved in the study |
| ☑ | Antibodies used |
| ☑ | For quantitative western blot assay, the following primary antibody was used: |
| ☑ | - sPDGFRβ polyclonal goat IgG antibody (R&D Systems Catalog #AF1042, Lot #GOV0418041). Membranes were incubated with 1 µg/mL of antibody overnight at room temperature, then incubated with donkey anti-goat IgG secondary antibody (Invitrogen, Cat #A15999, Lot #44-33-100114, 1:5,000 dilution) for 1 hour at room temperature. |
| ☑ | Validation |
| ☑ | Recombinant human sPDGFRβ protein (R&D Systems Catalog #385-PR/CF, Lot #RAM0714072) was used as a positive control in validating the antibodies. Consistently, the manufacturer's website specifies this antibody exhibits approximately 35% crossreactivity with recombinant human PDGFRβ. |
| ☑ | Human research participants |
| ☑ | Policy information about studies involving human research participants |
| Population characteristics | All participants (n=435) underwent neurological and neuropsychological evaluations performed using the Uniform Data Set and additional neuropsychological tests, and received a venipuncture for collection of blood for biomarker studies. A lumbar puncture was performed in 350 participants (81%) for collection of cerebrospinal fluid (CSF). The dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) for assessment of blood-brain barrier (BBB) permeability was performed in 245 participants (56%) that had no contraindications for contrast injection. Both LP and DCE-MRI were conducted in 172 participants. Among the 245 DCE-MRI participants, 74 and 96 were additionally studied for brain uptake of amyloid and tau PET radiotracers, respectively. - We studied 245 participants for MRI analysis with clinical dementia ratings of 0 and APOE3 genotype (n=130), 0.5 and APOE3 (n=14), 0 and APOE4 (n=76), and 0.5 and APOE4 (n=25), who exhibited 0 cognitive domain impairment, 1, or 2+ (see Extended Data Table 1 for additional information regarding age, sex, and education). - We studied 74 participants for amyloid PET analysis with clinical dementia ratings of 0 and APOE3 genotype (n=45), and 0 and APOE4 (n=29) (see Extended Data Table 2a for additional information regarding age, sex, and education). - We studied 96 participants for amyloid PET analysis with clinical dementia ratings of 0 and APOE3 genotype (n=60), and 0 and APOE4 (n=37) (see Extended Data Table 2b for additional information regarding age, sex, and education). - We studied 350 participants for CSF sPDGFRβ analysis with clinical dementia ratings of 0 and APOE3 genotype (n=157), 0.5 and APOE3 (n=40), 0 and APOE4 (n=105), and 0.5 and APOE4 (n=48), who exhibited 0 cognitive domain impairment, 1, or 2+ (see Extended Data Table 3 for additional information regarding age, sex, and education). |
| Recruitment | Potential participants were identified from the clinics, the community, existing research cohorts or self-referred applications. After an initial prescreening performed by the recruiters, the study coordinators assessed and confirmed the eligibility of the participants before planning and scheduling the procedures and the clinical assessments related to the study. |
The multi-modal approach for the identification of potential participants as well as the inclusion of participants from four different sites contributed to minimize the risk of self-selection bias. Furthermore, since the two main tests defining the grouping variables (i.e., the genotyping and the full neuropsychological assessment) were performed only after the enrollment of the participants, the bias related to the recruitment process potentially threatening the validity of the results is negligible. However, it should be noted that higher withdrawal rates were expected in the participants with mild cognitive dysfunction, potentially affecting the statistical power due to an unbalance between the cognitively normal participants and those with mild cognitive impairment.

**Ethics oversight**

University of Southern California (USC) maintains the complete program project and associated project Institutional Review Board (IRB) approvals reviewed and approved by USC IRB (Study IDs HS-16-00759 and HS-15-00875). All other sites also have local IRB approval. Banner University has IRB approval from WIRB (Study number 1173985). Huntington Medical Research Institute has approval from Quorum (QMR 33797/1). Mayo Clinic Arizona has approval from Mayo IRB (IRB#: 1709-97, Study ID PR1709-97-12). Washington University in St. Louis (WashU) has approval from the WashU Human Resources Protection Office (IRB ID#s: 201706100 and 201712150).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### 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 Methods section: Anatomical coronal spin echo T2-weighted scans were first obtained through the hippocampi (TR/TE 8020/50 ms, NEX = 1, slice thickness 2 mm with 2 mm gap between slices, FOV = 175 x 175 mm, matrix size = 448 x 448). Baseline coronal T1-weighted maps were then acquired using a T1-weighted 3D volumetric interpolated breath-hold sequence (VIBE) sequence and variable flip angle method using flip angles of 2°, 5°, 10°, 12°, and 15°. Coronal DCE-MRI covering the hippocampi and temporal lobes were acquired using a T1-weighted 3D VIBE sequence (FA = 15°, TR/TE = 5.14/2.18 ms, NEX = 1, slice thickness 5 mm with no gap, FOV 175 x 175 mm, matrix size 320 x 320, voxel size was 0.550 x 0.550 x 5 mm3). This sequence was repeated for a total of 16 min with an approximate time resolution of 15.4 sec. Gadolinium-based contrast agent (GBCA), 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 until the medial orbital frontal cortex. 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 | Not used |

#### Preprocessing

- **Preprocessing software**
  
  From Methods section and Supplementary Information:
  
  - **Blood-brain barrier permeability**:
    
    Post-processing analysis was performed using Rocketship software running with Matlab. The arterial input function (AIF), which was extracted from a region-of-interest (ROI) positioned at the internal carotid artery (ICA), was fitted with a bi-exponential function prior to fitting with the Patlak model. In a few cases when the ICA was not clearly visible a nearby large arterial vessel was used. The Patlak linearized regression mathematical analysis was used to generate the BBB permeability Ktrans maps, as we previously reported. The high spatiotemporal resolution allowed 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 small anatomical regions as thin as cortical gray matter areas. 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, Cissue (t), can be described as a function of the blood concentration, CI(af) (t), the intravascular blood volume, vp, and a blood-to-brain transfer constant, ktrans, that represents the flow from the intravascular to the extravascular extracellular space using equation (see Methods). A statistically significant intersubject variability in the measurement of vp 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 (e.g., enlarged ventricles, cortical atrophy, hippocampal shrinkage, etc.). Thus, the regional BBB Ktrans permeability were measured in 10 different gray matter ROIs including the hippocampus (HC), parahippocampal gyrus (PHG), caudate nucleus, thalamus, striatum, orbital frontal cortex (OFC), and inferior temporal gyrus (ITG), and white matter ROIs including subcortical watershed white matter fibers, corpus callosum, and internal capsule. 
  
  - **Volumetric analysis**:
    
    HC and PHG morphometry were performed using the FreeSurfer (v5.3.0) software package (http://surfer.nmr.mgh.harvard.edu/), as previously performed. HC and PHG were segmented using FreeSurfer Desikan-
Killiany and subcortical atlases. Then, regional volumes (mm³) were derived accordingly. The technical details of this procedure are described in previous publications. Data processing and visualization were performed using the Laboratory of Neuro Imaging (LONI) pipeline system (http://pipeline.loni.usc.edu) and Quantitative Imaging Toolkit.

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
- ROI-based
- Both

Anatomical location(s)
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 (e.g., enlarged ventricles, cortical atrophy, hippocampal shrinkage, etc.). Thus, the regional BBB Ktrans permeability were measured in 10 different gray matter ROIs including the hippocampus (HC), parahippocampal gyrus (PHG), caudate nucleus, thalamus, striatum, orbital frontal cortex (OFC), and inferior temporal gyrus (ITG), and white matter ROIs including subcortical watershed white matter fibers, corpus callosum, and internal capsule. 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

n/a Involved in the study
☐ Functional and/or effective connectivity
☒ Graph analysis
☒ Multivariate modeling or predictive analysis