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Endothelial-derived plasma exosome proteins in Alzheimer's disease angiopathy

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Abstract

Small cerebral vascular disease (SCeVD) demonstrated by white matter hyperintensity (WMH) on MRI contributes to the development of dementia in Alzheimer's disease (AD), but it has not been possible to correlate onset, severity, or protein components of SCeVD with characteristics of WMH in living patients. Plasma endothelial-derived exosomes (EDEs) were enriched by two-step immunoabsorption from four groups of participants with no clinical evidence of cerebrovascular disease: cognitively normal (CN) without WMH (CN without SCeVD, n = 20), CN with SCeVD (n = 22), preclinical AD (pAD) + mild cognitive impairment (MCI) without SCeVD (pAD/MCI without SCeVD, n = 22), and pAD/MCI with SCeVD (n = 16) for ELISA quantification of cargo proteins. Exosome marker CD81-normalized EDE levels of the cerebrovascular-selective biomarkers large neutral amino acid transporter 1 (LAT-1), glucose transporter type 1 (Glut-1), and permeability-glycoprotein (p-GP, ABCB1) were similarly significantly higher in the CN with SCeVD and pAD/MCI with SCeVD groups than their corresponding control groups without SCeVD. CD81-normalized EDE levels of Aβ40 and Aβ42 were significantly higher in the pAD/MCI with SCeVD group but not in the CN with SCeVD group relative to controls without SCeVD. Levels of normal cellular prion protein (PrPc), a receptor for amyloid peptides, and

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AUTHOR CONTRIBUTIONS

E.L. Abner, F.M. Elahi, E.J. Goetzl, and D. Kapogiannis contributed to the conception and design of the study; all authors contributed to the acquisition and analysis of patient and laboratory data; E.J. Goetzl, D. Kapogiannis, E.L. Abner, and F.M. Elahi contributed to initial drafting of the text.

CONFLICT OF INTEREST

EJG has filed an application with the US Patent and Trademark Office for the platform and methodologies described in this report. No other author has any potential conflict to report.

phospho-181T-tau were higher in both CN and pAD/MCI with SCeVD groups than in the corresponding controls. High EDE levels of Aβ40, Aβ42, and phospho-181T-tau in patients with WMH suggesting SCeVD appear at the pre-clinical or MCI stage of AD and therapeutic lowering of neurotoxic peptide levels may delay progression of AD angiopathy.

Keywords

amyloid; P-tau protein; prion cellular protein; small cerebral vascular disease

1 | INTRODUCTION

Patients with Alzheimer's disease (AD) have a high prevalence of cerebrovascular disease, small cerebral infarcts, and cerebral microhemorrhages which contribute to an increased incidence of dementia.[1-3] Cerebral vascular dysregulation and diminished cerebral blood flow are among the earliest pathophysiological changes of AD.[4,5] In contrast to the cerebral large vessel atherosclerosis underlying many ischemic strokes in elderly without AD, but with broadly prevalent vascular and metabolic risk factors, diverse forms of small cerebrovascular diseases predominate in AD.[6,7] Up to one-half of patients with AD have cerebral small vessel amyloid angiopathy and more than one-third of AD patients have cerebral small vessel arteriosclerosis at autopsy.[8-10] Several genome-wide association studies have demonstrated shared genetic risk factors involving abnormal cholesterol metabolism and immune responses for small cerebral vascular diseases (SCeVDs) and AD, but not for ischemic stroke from large cerebral vessel diseases and AD.[11] As presentation of SCeVD often precedes that of neuronal and other CNS cellular changes in AD, questions remain about whether early ischemia and hypoxia or failure of circulatory clearance of neurotoxic factors are primary events that contribute to the enhancement and spread of proteinopathic neurocellular disease.

A wide range of neuroimaging investigations, employing different MRI techniques and segmentation methods to quantify white matter hyperintensity (WMH), have confirmed and further elucidated the nature and pathogenic involvement of SCeVD in AD.[12] For example, global SCeVD assessed by the total of WMH volume plus volumes of small lacunar infarcts correlated with reductions in integrity of specific regions of corticocortical gray matter.[13] These findings suggested that SCeVD in AD is linked to the disruption of cortical gray matter networks. Contemporaneous application of the sophisticated methods of arterial spin-labeled MRI, 3DT1-MRI, and ethylcysteinate dimer single-photon emission computed tomography showed most significantly reduced cerebral blood flow in regions with the greatest atrophy, such as the medial temporal cortex, which in turn correlated with diminished language skills.[14] The results of such studies suggest that early SCeVD in AD damages the white matter structures and consequently disrupts the cortical gray matter networks. Other MRI studies of different design supported these conclusions that cortical lesions, such as microinfarcts and diffuse neuronal loss, develop in neuroanatomic and temporal relationships with SCeVD in AD.[15,16] Much of the vascular disturbance in early AD is attributable to capillary constriction by Aβ peptide stimulation of pericytes, that contract in response to released endogenous endothelin-1.[17] The sources of Aβ peptides and

other vasoactive factors, that damage small cerebral blood vessels and result in regional ischemia and enhanced effects of neurotoxins early in the course of AD, remain to be determined.

We now show that levels of the endothelial toxins Aβ40 and Aβ42 in plasma endothelial-derived exosomes (EDEs) of patients with preclinical AD (pAD) or mild cognitive impairment (MCI) with WMH by MRI are elevated relative to those with similar WMH who were cognitively normal (CN) and lacked evidence of AD and to those with pAD/MCI who lacked WMH.

2 | MATERIALS AND METHODS

2.1 | Study participants

Blood was obtained prospectively from research participants being evaluated in ongoing longitudinal studies of AD and cerebrovascular diseases at the Sanders-Brown Center on Aging of the University of Kentucky (UKy) and studies of brain aging at the Memory and Aging Center of the University of California, San Francisco (UCSF). No participants from either source had a history of stroke or transient ischemic attack or any clinical evidence of cerebrovascular disease. Further, none of the participants had been taking aspirin or a statin for at least 4 weeks prior to MRI and blood donation. Study protocols and procedures were approved by the UCSF and UKy Institutional Review Boards and were all conducted in accordance with the Code of Ethics of the World Medical Association. All study participants provided written informed consent.

The inclusion criteria (Table 1) for the 42 CN participants at UCSF and UKy encompassed intact daily functioning according to a reliable informant (CDR = 0) and other neuropsychological performances within normative standards including MMSE.[18] All had normal CSF levels of Aβ42 and P-T181-tau. The 38 participants at UKy and UCSF having preclinical AD (pAD) or MCI were classified according to the established criteria.[19,20] All were so defined by clinical neuropsychiatric evaluation and at least one of the following criteria (Table 1): (a) global Clinical Dementia Rating (CDR) = 0 (pAD) or CDR ≥ 0.5 (MCI) and/or Mini-Mental State Examination (MMSE) <28 (MCI), and (b) cerebrospinal fluid (CSF) level of Aβ42 <192 pg/mL and that of P-T181-tau >23 pg/mL.[21]

2.2 | MRI acquisition, processing, and analyses

Participants at UCSF and UKy were scanned with the same type of Siemens Prisma 3Tsystem and images were analyzed by the methods described.[22,23] All images at both UCSF and UKy were rated for burden of WMH by a board-certified neurologist in addition to being reviewed by a neuroradiologist to rule out other significant abnormalities. WMH volumes were obtained from T2-weighted FLAIR images using visual grading and an automated method for quantification and localization. Total WMH volume was estimated by summing all the voxels classified as WMH and normalized for total intracranial volume. Those with a modified Fazekas score ≥ 2 or with a ratio of WMH volume to total intracranial volume >0.22 were considered to have SCeVD, which included 22 for the CN set and 16 for the pAD/MCI set (Table 1). Those with a modified Fazekas score < 2 or with

a ratio of WMH volume to total intracranial volume ≤ 0.22 were considered to be free of significant SCeVD, which included 20 for the CN set and 22 for the pAD/MCI set.

2.3 | Enrichment of plasma EDEs and extraction of cargo proteins

At the time of enrollment in the current study and prior to any study treatments, blood was drawn from each participant for platelet-poor plasma preparation according to a published protocol.[24] Platelet-poor plasma samples were apportioned and stored in 0.25 mL aliquots at − 80°C. EDEs were precipitated with ExoQuick (System Biosciences, Menlo Park, CA), resuspended, and enriched by sequential immunoprecipitation with biotinylated anti-CD31 mouse monoclonal antibody (MEM-05, ThermoFisher Scientific) and then biotinylated anti-CD146 goat antibody (AF9-32, Biotechne, Novus Biologicals, Littleton, CO, USA) prior to lysis for quantification of cargo proteins by ELISAs.[22,24] Five percent of suspensions of enriched EDEs were saved prior to addition of detergent for counts and sizes using a Nanosight NS500 system with a G532nm laser module and NTA 3.1 nanoparticle tracking software (Malvern Instruments, Malvern, United Kingdom) as described.[24] To assess the association of biomarker proteins with exosomes in representative preparations of extracellular vesicles, 150 μL portions of suspensions of intact EDEs were precipitated with 1 ug of biotinylated anti-human CD81 antibody (M38, Abcam, Cambridge, MA) in 25 μL of 3% BSA plus 10 μL of streptavidin-agarose Ultralink resin (ThermoFisher Scientific) in 40 μL of 3% BSA, and incubation for 30 minutes at room temperature with mixing. After centrifugation, acetic acid release of EDEs, recentrifugation, neutralization of supernatant, detergent lysis of free EDEs as for the initial enrichment procedure, and adjustment of volume to 150 μL, these preparations were analyzed by selected ELISAs in parallel with primary preparations of EDEs.

Proteins in extracts of EDEs and CSF were quantified with human-specific ELISAs for prion cellular protein (PrPc), glucose transporter type 1 (Glut-1), permeability-glycoprotein (p-gp, ABCB1), and the tetraspanning exosome marker CD81(Cusabio-American Research Products, Waltham, MA, USA), the large neutral amino acid transporter 1 (LAT-1) (Cloud-Clone Corp.-American Research Products), phospho-threonine181-tau (P-T181-tau) (FUJIREBIO, US, Inc, Malvern, PA), and Aβ42 (ultrasensitive) and Aβ40 (Invitrogen, ThermoFisher Scientific, Vienna, Austria). All EDE enrichments and ELISAs were performed by one investigator (EJG).

2.4 | Statistical analyses

The Shapiro-Wilks test showed that data in each set, except for four, were distributed normally. Groups of data were compared using an unpaired Student's *t* test, including a Bonferroni correction (Prism 7; GraphPad Software, La Jolla, CA, USA). For the four nonnormally distributed sets, significance was determined by a Mann-Whitney *U* test. We performed age- and sex-adjusted linear regressions to confirm results and ensure that findings were not explained by confounding due to age and sex.

3 | RESULTS

The four groups were similar for sex and means of age and education with the exception of distribution of sex in the two CN subgroups (Table 1). None of the participants in any group had a history of stroke, transient ischemic attack or any other clinical evidence of cerebrovascular disease. Tests of neuropsychiatric function, cognition and CSF proteins established the characteristics of the two pAD and MCI groups, that were considered together.

Levels of the CD81 exosome marker were similar to those found previously in two immunoabsorption step preparations of EDEs and were indistinguishable statistically among the groups (Figure 1).[22,24] EDE Nanosight counts (mean ± SEM) supported the lack of differences between CD81 levels for the CN without SCeVD, CN with SCeVD, pAD/MCI without SCeVD and pAD/ MCI with SCeVD groups, respectively, at $6.8 \pm 0.6 \times 10^9$/mL, $7.2 \pm 0.7 \times 10^9$/mL, $8.1 \pm 0.8 \times 10^9$/mL, and $7.5 \pm 0.8 \times 10^9$/mL. EDE sizes by Nanosight also were indistinguishable for the four groups at 110 ± 18 nm (mean ± SEM), 115 ± 24 nm, 96 ± 16 nm and 116 ± 22 nm, respectively. LAT-1, Glut-1, and P-gp, the three protein biomarkers expressed exclusively in cerebrovascular EDEs,[25-27] also were found in the current EDE preparations at CD81-normalized levels observed previously. Further, the CD81-normalized levels of all three of these cerebrovascular EDE markers were similarly significantly higher in the groups with greater WMH, as an indication of SCeVD, than their corresponding control groups without SCeVD for both CN and pAD/MCI sets (Figure 1). This finding supports the presence of cerebrovascular endothelial perturbation and/or injury of the same severity in both groups of subjects with SCeVD relative to their respective control groups without SCeVD. CD81-expressing extracellular vesicles, presumed to be exosomes, were isolated by CD81 immunoabsorption from six primary preparations each of the CN and pAD/MCI sets with SCeVD for comparative ELISA quantification of LAT-1 and Glut-1 before and after further immunoisolation of exosomes. The mean ± SEM levels of LAT-1 were 1523 ± 192 and 1561 ± 289 pg/mL in the primary and 1264 ± 141 and 1300 ± 243 pg/mL in CD81-selected exosome (83% and 83% of primary) preparations for the CN and pAD/MCI sets, respectively. The mean ± SEM levels of Glut-1 were 1088 ± 66.9 and 1164 ± 85.3 pg/mL in the primary and 855 ± 92.0 and 863 ± 67.4 pg/mL in CD81-selected exosome (79% and 74% of primary) preparations for the CN and pAD/MCI sets, respectively.

The EDE levels of Aβ40 and Aβ42 protein markers of amyloid angiopathy were significantly higher in the pAD/MCI with SCeVD group than the control pAD/MCI without SCeVD group, but not in the CN with SCeVD group than the CN without SCeVD group (Figure 2). In contrast, CD81-normalized EDE levels of the putative amyloid peptide-binding protein PrPc and P-181T-tau were significantly elevated in both the CN with SCeVD and pAD/MCI with SCeVD groups relative to their respective control groups without SCeVD. This pattern of increased expression of PrPc and P-181T-tau resembles that of the three markers of cerebrovascular endothelial injury (Figure 1). The pattern of increased expression of Aβ40 and Aβ42 proteins, however, requires concomitant presence of SCeVD and pAD/MCI. Further, the EDE levels of P-181T-tau for both CN groups (Figure 2D) were significantly higher than those of their corresponding pAD/MCI groups.

4 | DISCUSSION

CD81-normalized levels of the cerebrovascular-specific proteins Glut-1, LAT-1, and P-gp in endothelial cell-derived plasma exosomes (EDEs) were significantly higher in both the CN and pAD/MCI groups having SCeVD, as assessed by high MRI levels of WMH, as compared with the corresponding CN and pAD/MCI groups not having SCeVD by the same WMH criteria (Figure 1). It is assumed that higher plasma EDE levels of these cerebrovascular-specific proteins in all participants with SCeVD reflect their elevated levels in damaged endothelial cells of CNS microvasculature, as there was no evidence of other cerebrovascular lesions. This assumption is also based on the findings that levels of cargo proteins in CNS neuron-derived plasma exosomes (NDEs) reflect the levels in CNS neurons in the chronic state of AD.[28-30] Here the plasma levels of EDEs were the same for each group as for NDEs in established AD based on counts and quantities of the exosome marker CD81 (Figure 1). Only in the rapidly changing states of acute traumatic brain injury, where plasma levels of NDEs are altered significantly, may plasma NDE and EDE levels of cargo proteins not accurately represent those in CNS neurons and endothelial cells, respectively.[31,32]

Significant increases in EDE levels of the amyloid-related peptides Aβ40 and Aβ42 were found exclusively in pAD/MCI with SCeVD subjects relative to AD controls, but not in CN with SCeVD subjects relative to CN without SCeVD controls (Figure 2), which supports the possibility that higher EDE levels of these proteins are indicative of amyloid microangiopathy early in pAD and MCI. In contrast to the requirements of both pAD or MCI and SCeVD for increased expression of Aβ40 and Aβ42, P-181T-tau and PrPc levels in EDEs were higher in both CN with SCeVD and pAD/MCI with SCeVD subjects suggesting a sole necessity for SCeVD, but not pAD/MCI.

Cerebral amyloid angiopathy (CAA) appears to be attributable predominantly to the pathogenic processes that parallel those inducing neuronal damage in AD.[33] CAA is present in three-times more individuals with AD than matched CN controls. Distinctive neuropathology evoked by CAA includes microhemorrhages, microinfarcts, and vascular blood-brain barrier leakage.[34] Induction of cerebrovascular remodeling characterized by loss of alpha-smooth muscle actin, elastin, and collagen is induced by deposition of helical filament P-tau in intra-parenchymal small arteries and arterioles, and is separate from and often precedes CAA.[35] Synergistic effects of Abeta and P-tau in SCeVD were suggested by prevention of Abeta-induced synaptic dysfunction in the absence of P-tau in an animal model.[36] That P-tau aggregates in cerebral vasculature are concentrated at endothelial cells and smooth muscle cells suggested that P-tau would be detected in EDEs. Indeed, EDE levels of P-tau were significantly elevated in subjects with WMH suggesting SCeVD whether CN or affected by pAD/MCI (Figure 2D).

PrPc is most highly expressed in the central nervous system in humans and its levels decrease in some areas of the brain, such as the hippocampus, with aging and neurodegenerative diseases.[37-39] The capacity of PrPc to bind amyloid polypeptide Aβ42, with especially high affinity for the oligomeric form Aβ42o, has been shown in direct binding studies as well as by both resultant interactions with other proteins and neuronal

biochemical and functional responses.[40,41] The complex structural determinants of binding of Aβ42 by PrPc, the interactions between the amino-terminal and carboxyl-terminal domains of PrPc which require flexibility of the hydrophobic joining segment, and the neurotoxic effects of substituent sequences of the intrinsically disordered amino-terminus of PrPc are worthy of further studies.

Limitations of this study include possibilities of misclassification of some subjects by neuroimaging and/or neurocognitive evaluations which would influence the results. Further, participants were highly educated and primarily of a high socioeconomic status. Findings we report thus may differ for other populations.

The extent and severity of amyloid microangiopathy in AD, which contributes to the pathogenesis of dementia, may be prevented or even reversed by the therapeutic modalities that reduce the Aβ peptide levels in brain or their binding to and entry into CNS neurons. The development of CAA in APP Dutch mice has recently been prevented by early combined reduction of Aβ40 and Aβ42.[42] Early cerebral amyloid microangiopathy in pAD may respond to agents that have access to the microvasculature but not CNS neurons. For example, intravenously administered blocking antibodies to PrPc would be expected to reach endothelial and other vascular cells and thereby prevent amyloid peptide binding to and effects on these cells, but not as fully access CNS neurons. Diminishing cerebral amyloid microangiopathy in some patients may significantly prevent dementia.

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Abbreviations:

Aβ	beta-amyloid protein
AD	Alzheimer's disease
CN	cognitively normal
CDR	clinical dementia rating
EDE	endothelial-derived exosome
Glut-1	glucose transporter type 1
LAT-1	large neutral amino acid transporter 1
MCI	mild cognitive impairment
MMSE	mini-mental state examination

P-181T-tau	phospho-threonine181-tau
pAD	pre-AD
pGP	permeability-glycoprotein
PrPc	normal cellular prion protein
SCeVD	small cerebral vascular disease
WMH	white matter hyperintensity

REFERENCES

1. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. JAMA. 1997;277:813–817. [PubMed: 9052711]
2. Esiri MM, Nagy Z, Smith MZ, Barnetson L, Smith AD. Cerebrovascular disease and threshold for dementia in the early stages of Alzheimer's disease. Lancet. 1999;354:919–920. [PubMed: 10489957]
3. Snowdon DA, Nun S. Healthy aging and dementia: findings from the Nun Study. Ann Intern Med. 2003;139:450–454. [PubMed: 12965975]
4. Iturria-Medina Y, Sotero RC, Toussaint PJ, et al. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. Nat Commun. 2016;7:11934. [PubMed: 27327500]
5. Love S, Miners JS. Cerebrovascular disease in ageing and Alzheimer's disease. Acta Neuropathol. 2016;131:645–658. [PubMed: 26711459]
6. Roher AE, Esh C, Rahman A, Kokjohn TA, Beach TG. Atherosclerosis of cerebral arteries in Alzheimer disease. Stroke. 2004;35:2623–2627. [PubMed: 15375298]
7. Beach TG, Wilson JR, Sue LI, et al. Circle of Willis atherosclerosis: association with Alzheimer's disease, neuritic plaques and neurofibrillary tangles. Acta Neuropathol. 2007;113:13–21. [PubMed: 17021755]
8. Ellis RJ, Olichney JM, Thal LJ, et al. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. Neurology. 1996;46:1592–1596. [PubMed: 8649554]
9. Kalaria RN. Small vessel disease and Alzheimer's dementia: pathological considerations. Cerebrovasc Dis. 2002;13(suppl 2):48–52. [PubMed: 11901243]
10. De Reuck J, Deramecourt V, Cordonnier C, Leys D, Maurage CA, Pasquier F. The impact of cerebral amyloid angiopathy on the occurrence of cerebrovascular lesions in demented patients with Alzheimer features: a neuropathological study. Eur J Neurol. 2011;18:913–918. [PubMed: 21244582]
11. Traylor M, Adib-Samii P, Harold D, et al. Shared genetic contribution to ischaemic stroke and Alzheimer's disease. Ann Neurol. 2016;79:739–747. [PubMed: 26913989]
12. Frey BM, Petersen M, Mayer C, Schulz M, Cheng B, Thomalla G. Characterization of white matter hyperintensities in large-scale MRI-studies. Front Neurol. 2019;10:238. [PubMed: 30972001]
13. Nestor SM, Misic B, Ramirez J, et al. Small vessel disease is linked to disrupted structural network covariance in Alzheimer's disease. Alzheimers Dement. 2017;13:749–760. [PubMed: 28137552]
14. Huang CW, Hsu SW, Chang YT, et al. Cerebral perfusion insufficiency and relationships with cognitive deficits in Alzheimer's disease: a multiparametric neuroimaging study. Sci Rep. 2018;8:1541. [PubMed: 29367598]
15. Peres R, De Guio F, Chabriat H, Jouvent E. Alterations of the cerebral cortex in sporadic small vessel disease: a systematic review of in vivo MRI data. J Cereb Blood Flow Metab. 2016;36:681–695. [PubMed: 26787108]

16. Blair GW, Doubal FN, Thrippleton MJ, Marshall I, Wardlaw JM. Magnetic resonance imaging for assessment of cerebrovascular reactivity in cerebral small vessel disease: a systematic review. J Cereb Blood Flow Metab. 2016;36:833–841. [PubMed: 26884471]
17. Nortley R, Korte N, Izquierdo P, et al. Amyloid beta oligomers constrict human capillaries in Alzheimer's disease via signaling to pericytes. Science. 2019;365:eaav9518. [PubMed: 31221773]
18. Kramer JH, Jurik J, Sha SJ, et al. Distinctive neuropsychological patterns in frontotemporal dementia, semantic dementia, and Alzheimer disease. Cogn Behav Neurol. 2003;16:211–218. [PubMed: 14665820]
19. Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol. 2007;6:734–746. [PubMed: 17616482]
20. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the pre-clinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7:280–292. [PubMed: 21514248]
21. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009;65:403–413. [PubMed: 19296504]
22. Elahi FM, Casaletto KB, Altendahl M, et al. “Liquid biopsy” of white matter hyperintensity in functionally normal elders. Front Aging Neurosci. 2018;10:343. [PubMed: 30483114]
23. Al-Janabi OM, Brown CA, Bahrani AA, et al. Distinct white matter changes associated with cerebrospinal fluid amyloid-beta1-42 and hypertension. J Alzheimers Dis. 2018;66:1095–1104. [PubMed: 30400099]
24. Goetzl EJ, Schwartz JB, Mustapic M, et al. Altered cargo proteins of human plasma endothelial cell-derived exosomes in atherosclerotic cerebrovascular disease. FASEB J. 2017;31:3689–3694. [PubMed: 28476896]
25. Seidner G, Alvarez MG, Yeh JI, et al. GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain barrier hexose carrier. Nat Genet. 1998;18:188–191. [PubMed: 9462754]
26. Boado RJ, Li JY, Nagaya M, Zhang C, Pardridge WM. Selective expression of the large neutral amino acid transporter at the blood-brain barrier. Proc Natl Acad Sci U S A. 1999;96:12079–12084. [PubMed: 10518579]
27. Schinkel AH. P-Glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev. 1999;36:179–194. [PubMed: 10837715]
28. Fiandaca MS, Kapogiannis D, Mapstone M, et al. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. Alzheimers Dement. 2015;11:600–607.e601. [PubMed: 25130657]
29. Goetzl EJ, Boxer A, Schwartz JB, et al. Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. Neurology. 2015;85:40–47. [PubMed: 26062630]
30. Goetzl EJ, Boxer A, Schwartz JB, et al. Low neural exosomal levels of cellular survival factors in Alzheimer's disease. Ann Clin Transl Neurol. 2015;2:769–773. [PubMed: 26273689]
31. Goetzl EJ, Elahi FM, Mustapic M, et al. Altered levels of plasma neuron-derived exosomes and their cargo proteins characterize acute and chronic mild traumatic brain injury. FASEB J. 2019;33:5082–5088. [PubMed: 30605353]
32. Goetzl EJ, Peltz CB, Mustapic M, Kapogiannis D, Yaffe KC. Neuron-derived plasma exosome proteins after remote traumatic brain injury. J Neurotrauma. 2020;37:382–388. [PubMed: 31441374]
33. Greenberg SM, Bacskai BJ, Hernandez-Guillamon M, Pruzin J, Sperling R, van Veluw SJ. Cerebral amyloid angiopathy and Alzheimer disease—one peptide, two pathways. Nat Rev Neurol. 2020;16:30–42. [PubMed: 31827267]
34. Freeze WM, Bacskai BJ, Frosch MP, et al. Blood-brain barrier leakage and microvascular lesions in cerebral amyloid angiopathy. Stroke. 2019;50:328–335. [PubMed: 30661497]
35. Merlini M, Wanner D, Nitsch RM. Tau pathology-dependent remodelling of cerebral arteries precedes Alzheimer's disease-related microvascular cerebral amyloid angiopathy. Acta Neuropathol. 2016;131:737–752. [PubMed: 26988843]

36. You Y, Perkins A, Cisternas P, et al. Tau as a mediator of neurotoxicity associated to cerebral amyloid angiopathy. Acta Neuropathol Commun. 2019;7:26. [PubMed: 30808415]

37. Castle AR, Gill AC. Physiological functions of the cellular prion protein. Front Mol Biosci. 2017;4:19. [PubMed: 28428956]

38. Wulf MA, Senatore A, Aguzzi A. The biological function of the cellular prion protein: an update. BMC Biol. 2017;15:34. [PubMed: 28464931]

39. Whitehouse IJ, Jackson C, Turner AJ, Hooper NM. Prion protein is reduced in aging and in sporadic but not in familial Alzheimer's disease. J Alzheimers Dis. 2010;22:1023–1031. [PubMed: 20930299]

40. Smith LM, Kostylev MA, Lee S, Strittmatter SM. Systematic and standardized comparison of reported amyloid-beta receptors for sufficiency, affinity, and Alzheimer's disease relevance. J Biol Chem. 2019;294:6042–6053. [PubMed: 30787106]

41. Jarosz-Griffiths HH, Corbett NJ, Rowland HA, et al. Proteolytic shedding of the prion protein via activation of metallopeptidase ADAM10 reduces cellular binding and toxicity of amyloid-beta oligomers. J Biol Chem. 2019;294:7085–7097. [PubMed: 30872401]

42. Schelle J, Wegenast-Braun BM, Fritschi SK, et al. Early Abeta reduction prevents progression of cerebral amyloid angiopathy. Ann Neurol. 2019;86:561–571 [PubMed: 31359452]

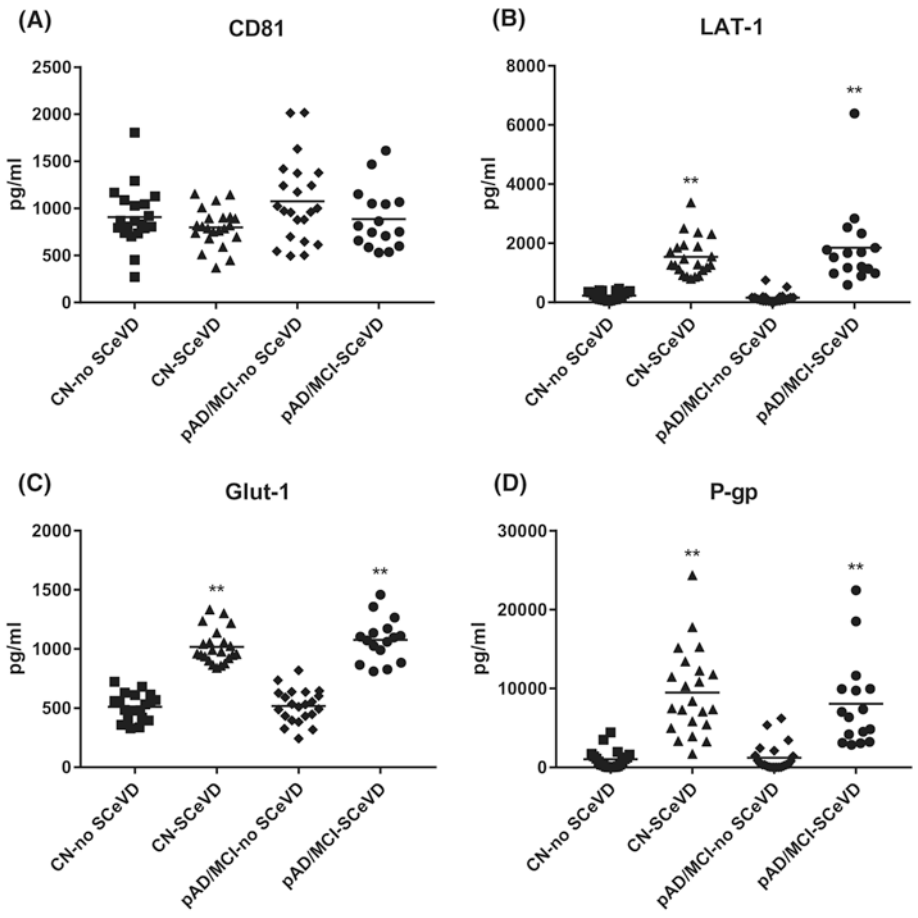

FIGURE 1.

EDE levels of cerebrovascular-selective cargo proteins in cognitively normal group without SCeVD (CN-no SCeVD), cognitively normal group with SCeVD (CN-SCeVD), pAD/MCI group without SCeVD (pAD/MCI-no SCeVD), and pAD/MCI group with SCeVD (pAD/MCI-SCeVD). Each point represents the value for one participant and the horizontal line in point clusters is the mean level for that group. Mean ± SEM for CN-no SCeVD, CN-SCeVD, pADMCI-no SCeVD, and pADMCI-SCeVD, respectively, are 906 ± 24.6, 799 ± 43.6, 1076 ± 93.7, and 886 ± 81.0 pg/mL for CD81 (A), 232 ± 32.0, 1535 ± 140, 154 ± 36.5, and 1847 ± 341 pg/mL for type 1 large neutral amino acid transporter (LAT-1) (B), 510 ± 25.1, 1018 ± 30.7, 517 ± 30.0, and 1078 ± 45.6 pg/mL for type 1 glucose transporter (Glut-1) (C), and 1040 ± 266, 9499 ± 1167, 1204 ± 372, and 8056 ± 1417 pg/mL for permeability-glycoprotein (P-gp) (D). The significance of differences shown between values for CN-no SCeVD and CN-SCeVD and between values for pAD/MCI-no SCeVD and pAD/MCI-SCeVD, respectively, were calculated by an unpaired Student's *t* test; $^{**}P < .0001$

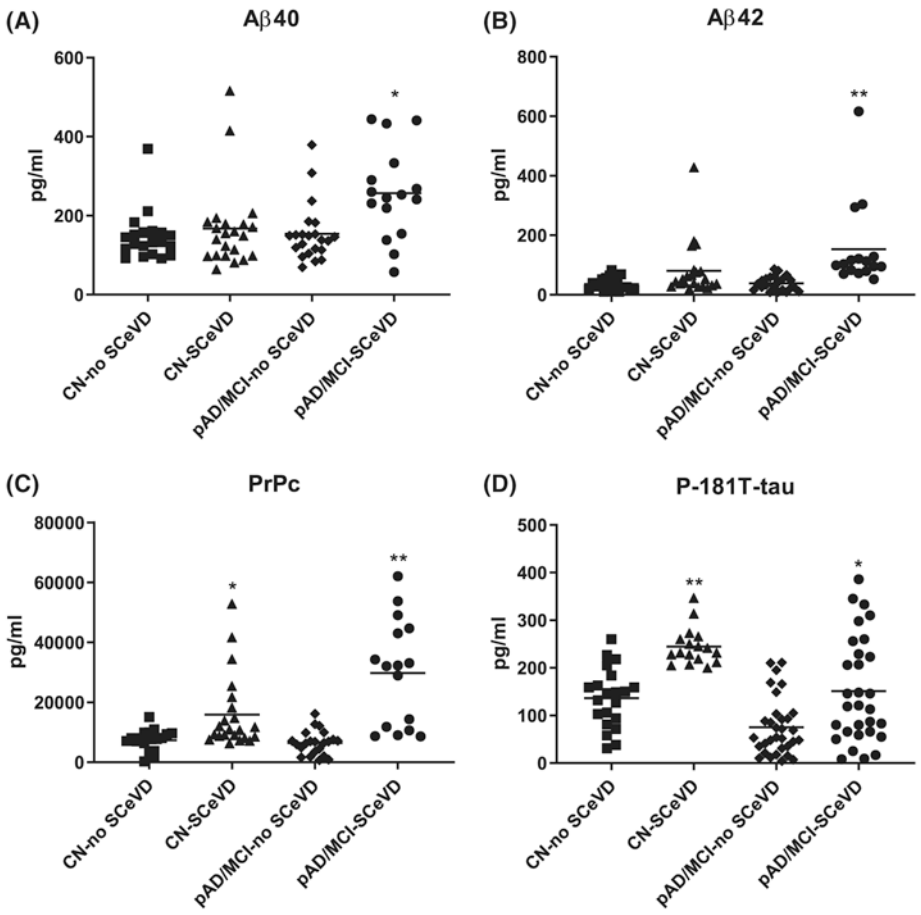

FIGURE 2.

EDE levels of amyloid peptides and the putative amyloid peptide receptor protein PrPc in cognitively normal group without SCeVD (CN-no SCeVD), cognitively normal group with SCeVD (CN-SCeVD), pAD/MCI group without SCeVD (pAD/MCI-no SCeVD), and pAD/MCI group with SCeVD (pAD/MCI-SCeVD). Each point represents the value for one participant and the horizontal line in point clusters is the mean level for that group. Mean ± SEM for CN-no SCeVD, CN-SCeVD, pAD/MCI-no SCeVD and pAD/MCI-SCeVD, respectively, are 146 ± 13.7, 167 ± 22.6, 154 ± 15.5, and 257 ± 28.7 pg/mL for Aβ40 (A), 35.8 ± 4.77, 74.6 ± 19.4, 38.2 ± 5.04, and 153 ± 35.8 pg/mL for Aβ42 (B), 7376 ± 774, 15,899 ± 2636, 6461 ± 851, and 29,797 ± 4417 pg/mL for PrPc (C), and 136 ± 13.6, 245 ± 9.5, 75.3 ± 10.7 (n = 32), and 151 ± 20.1 (n = 30) pg/mL for P-181T-tau (D). The significance of differences shown between values for CN-no SCeVD and CN-SCeVD and between values for pAD/MCI-no SCeVD and pAD/MCI-SCeVD, respectively, were calculated by an unpaired Student's *t* test; $*P < .01$, $**P < .001$

TABLE 1

Participant demographics and clinical characteristics

					Evidence for pAD/MCI		
Group	**N**	**Age (mean ± SEM)**	**Gender (F/M)**	**Education (years, mean ± SEM)**	**MCI**	**Abnormal MMSE&/or CDR**	**Abnormal CSF P-T181-tau or Aβ42**
CN without WMH	20	74 ± 1.4	9/11	18 ± 0.83	0	0	0
CN with WMH	22	73 ± 1.7	13/9	18 ± 0.42	0	0	0
pAD/MCI without WMH	22	73 ± 1.2	10/6	17 ± 0.51	10	12	17
pAD/MCI with WMH	16	76 ± 1.3	11/9	17 ± 0.46	6	6	13

Abbreviations: CDR, clinical dementia rating; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; WMH, white matter hyperintensity; MMSE, mini-mental state examination; pAD, pre-clinical Alzheimer's disease.