RESEARCH ARTICLE

Vascular endothelial-cadherin as a marker of endothelial injury in preclinical Alzheimer disease

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Abstract

Objective: Endothelial dysfunction is an early and prevalent pathology in Alzheimer disease (AD). We here investigate the value of vascular endothelial-cadherin (VEC) as a cerebrospinal fluid (CSF) marker of endothelial injury in preclinical AD. **Methods:** Cognitively normal participants (Clinical Dementia Rating [CDR] 0) from the Knight Washington University-ADRC were included in this study ($n = 700$). Preclinical Alzheimer's Cognitive Composite (PACC) scores, CSF VEC, tau, p-tau181, Aβ42/Aβ40, neurofilament light-chain (NFL) levels, and magnetic resonance imaging (MRI) assessments of white matter injury (WMI) were obtained from all participants. A subset of participants underwent brain amyloid imaging using positron emission tomography (amyloid-PET) ($n = 534$). Linear regression examined associations of CSF VEC with PACC and individual cognitive scores in preclinical AD. Mediation analyses examined whether CSF VEC mediated effects of CSF amyloid and tau markers on cognition in preclinical AD. **Results:** CSF VEC levels significantly correlated with PACC and individual cognitive scores in participants with amyloid (A+T±N±; $n = 558$) or those with amyloid and tau pathologies (A+T+N ±; $n = 259$), after adjusting for covariates. CSF VEC also correlated with CSF measures of amyloid, tau, and neurodegeneration and global amyloid burden on amyloid-PET scans in our cohort. Importantly, our findings suggest that CSF VEC mediates associations of CSF Aβ42/Aβ40, p-tau181, and global amyloid burden with cognitive outcomes in preclinical AD. **Interpretation:** Our results support the utility of CSF VEC as a marker of endothelial injury in AD and highlight the importance of endothelial injury as an early pathology that contributes to cognitive impairment in even the earliest preclinical stages.

Introduction

Alzheimer disease (AD) is the most common cause of dementia in individuals above the age of 65 and a major cause of mortality and morbidity among the elderly.[1] The abnormal aggregation and deposition of amyloid and tau proteins, in the form of amyloid plaques and neurofibrillary tangles (respectively), are the major pathological hallmarks of AD.[2] Proposed AD models suggest that amyloid aggregation is the earliest pathology in AD that begins a decade or longer prior to symptom onset, a stage referred to as "preclinical AD."[3] Amyloid aggregation is followed by progressive tau accumulation in vulnerable brain regions ultimately leading to significant synaptic and neuronal loss and the onset of cognitive impairment (i.e., the onset of "symptomatic" AD).[3] As therapeutic interventions targeting amyloid and tau have not been successful in preventing or reducing AD progression, there has been increasing interest in identifying early pathological substrates of AD, beyond amyloid and tau, which contribute to neurodegeneration.[4,5]

Endothelial dysfunction refers to characteristic structural or functional alterations at the intracellular or cell membrane level which interfere with endothelial cell integrity or function.[6–14] Endothelial dysfunction is a prevalent pathological substrate of AD, as it is observed in ≥90% of AD brains at autopsy,[9,15] including those with only mild degrees of amyloid deposition. Endothelial abnormalities in AD often occur in the absence of cerebrovascular pathology (i.e., atherosclerosis or arteriolosclerosis) or cerebral amyloid angiopathy. Importantly, emerging evidence from animal and clinical studies suggests that endothelial dysfunction may precede amyloid pathology in early AD[16,17] and directly contribute to synaptic loss and

cognitive impairment independently of amyloid or tau.[18–20] However, our understanding of the role of endothelial injury in clinical studies of AD has been limited by the absence of reliable endothelial biomarkers.

We here identify vascular endothelial-cadherin (VE-cadherin, VEC; cadherin-5) as a potential novel marker of endothelial injury in AD using cerebrospinal fluid (CSF) studies and transcriptomic analyses of AD brains in a large well-characterized cohort of cognitively normal individuals with preclinical AD. VEC is an abundant endothelium-specific adhesion molecule and a key component of endothelial tight junctions.[21–23] In this study, we aim to elucidate the relationship between endothelial injury and cognition in the earliest preclinical stages of disease utilizing CSF VEC as a marker of endothelial injury. We will examine the associations of CSF VEC, Aβ42/Aβ40, p-tau181, tau, and neurofilament light-chain (NFL) levels with global and domain-specific (i.e., episodic memory, language, executive function, and orientation) preclinical cognitive outcomes using the Preclinical Alzheimer's Cognitive Composite (PACC) in a large ($n = 700$) well-characterized cohort of cognitively normal individuals (Clinical Dementia Rating [CDR] of 0), including those with biomarker evidence of preclinical AD pathology ($n = 558$) (i.e., those with biomarker evidence of amyloid pathology with or without biomarker evidence of tau). We will also examine the relationship between CSF VEC and CSF markers of amyloid, tau, and neurodegeneration in preclinical AD. Finally, we will investigate whether endothelial injury mediates the effects of amyloid and tau pathology on cognitive outcomes in the early preclinical stages.

We herein show – for the first time – that CSF VEC levels are increased in preclinical AD and correlate with

cognitive outcomes and CSF markers of amyloid, tau, and neurodegeneration. Furthermore, our data suggest that CSF VEC levels, reflective of endothelial injury, mediate the relationship between amyloid or tau pathology and cognition in preclinical AD. Together, our findings support the utility of CSF VEC as a novel surrogate of endothelial injury in preclinical AD and highlight the importance of endothelial dysfunction as an early and prominent pathological substrate of AD which contributes to cognitive impairment in even the earliest preclinical stages.

Methods

Study participants

Participants ($n = 700$) were community-dwelling volunteers enrolled in longitudinal studies of healthy aging and dementia (i.e., the Memory and Aging Project) at the Knight-Alzheimer Disease Research Center (Knight-ADRC), Washington University School of Medicine. Participants were in good general health with no other medical illness that could contribute to dementia and no contraindication to lumbar puncture (LP) or magnetic resonance imaging (MRI). Apolipoprotein E (*APOE*) genotypes were obtained as described.[24]

Participants from the Knight-ADRC were included in this study if they were cognitively normal as defined by a CDR of 0 and had the following: (i) detailed cognitive assessments including PACC scores; (ii) CSF biomarker measurements for VEC, tau, p-tau181, Aβ42, Aβ40, and NFL; and (iii) structural brain MRI assessments. Participants with a history of traumatic brain injury, symptomatic cerebrovascular disease (i.e., stroke or intracranial hemorrhage), or those with significant cerebral small vessel disease were excluded. A subset of participants ($n = 534$) had in vivo brain amyloid imaging using positron emission tomography (PET). Baseline cognitive, MRI, or PET assessments were those closest to the time of the LP.

Studies were approved by the Human Research Protection Office at Washington University. Written informed consent was obtained from all participants.

Cognitive assessments

All participants in this study ($n = 700$) were cognitively normal individuals as defined by a CDR of 0. PACC is a continuous measure of global cognition, which is sensitive to change in preclinical AD, and has been implemented in several preclinical AD cohorts with slight variations in calculation methods across different centers.[25,26] The Knight-ADRC PACC scores used in this study consisted of scores for four tests representing different cognitive domains: episodic memory (free recall score from the Free and Cued Selective Reminding Test [FCSRT-Free]),[27] mental status and orientation (total score from the Mini-Mental Status Examination [MMSE]),[28] executive functions (Digit-Symbol Substitution test of the Weschler Adult Intelligence Scale-Revised),[29,30] and verbal semantic memory (category fluency for animals [Animal Fluency]).[31] Scores for each of these four tests were standardized to *z* scores. The PACC score for each participant was calculated as the average *z* score of all four domains. PACC scores were obtained from all participants ($n = 700$).

CSF collection, storage, and processing

CSF samples (20–30 mL) were collected from all participants[32] and analyzed for total tau, p-tau181, Aβ42, and Aβ40 levels by enzyme-linked immunosorbent assays (Fujirebio, Japan).[33,34] CSF NFL levels were measured using a commercial immunoassay (Uman Diagnostics, Sweden).

CSF VEC levels were measured using the SomaScan® 1.3k platform (Somalogic) that utilizes slow off-rate modified aptamers and provides highly reproducible measurements of proteins in biological fluids. SomaScan has been successfully implemented in similar AD cohorts[35] and provides higher test–retest reproducibility and sensitivity for protein quantification than traditional antibody-based or mass spectrometry approaches.[36] We have shown that SomaScan and traditional immunoassay-based methods provide comparable results for CSF biomarker measurements.[37] All CSF biomarker measures were standardized to *z* scores prior to analyses.

ATN classification

Participants in our study cohort (CDR 0, $n = 700$) were classified according to the National Institute of Aging-Alzheimer's Association (NIA-AA) "ATN" classification for the presence of amyloid, tau, and neurodegeneration[38] using the following cutoff values for CSF biomarkers: Aβ42/Aβ40 ≤ 0.10 for A+, p-tau181 ≥ 52 pg/mL for T+, and tau ≥375 pg/mL for N+.[39–42] In the ATN classification, amyloid positivity alone denotes AD-pathological change while individuals with both amyloid and tau positivity meet the biological definition of AD[38]. Of the $n = 700$ participants, $n = 558$ were classified as amyloid-positive (A+T±N±) and $n = 142$ as amyloid-negative (A−T±N±). Of the amyloid-positive participants ($n = 558$), $n = 259$ also had evidence of tau pathology and therefore met the biological definition of AD (A+T+N±). Of the amyloid-negative cohort ($n = 142$),

$n = 106$ participants were A-T-N- and therefore were considered biomarker-confirmed healthy controls.

Magnetic resonance imaging

Structural MRI was performed using a 3.0 or 1.5 T scanner (Data S1). Preprocessed data from cortical reconstruction and volumetric segmentation of T1 images using the image analysis suite FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) were available for all study participants.[43–45] FreeSurfer measurements of white matter hypo-intensity were normalized to intracranial volumes (ICV), standardized to z scores, and adjusted for as a covariate in all analyses as a measure of white matter injury (WMI). Previous studies have shown the comparability of FreeSurfer measurements of white matter hypo-intensities obtained from T1 images with estimates of white matter hyperintensities obtained from FLAIR images using the Statistical Parametric Mapping-Lesion Segmentation Toolbox (SPM-LST) as a measure of WMI in cognitively normal elderly.[46]

In vivo amyloid imaging

In vivo brain amyloid PET imaging using the amyloid ligand Pittsburgh Compound B (PiB; $n = 377$) or 18F-AV-45 ($n = 157$) was obtained in a subset of participants ($n = 534$).[47–50] Under the standard protocol, quantitative PET analysis uses 30–60 min postinjection as the time window for PiB and 50–70 min for 18F-AV-45. FreeSurfer calculations of partial volume-corrected standardized uptake value ratios (SUVRs) were obtained.[48] The mean cortical SUVR, which is the arithmetic mean of SUVRs from the precuneus, prefrontal cortex, gyrus rectus, and lateral temporal regions, was used as a measure of global amyloid burden.[49] PET-amyloid positivity was defined using a cutoff value for mean cortical amyloid SUVR of ≥ 1.42.[51] The Knight-ADRC has developed and validated equations for harmonization of amyloid PET data between amyloid tracers based on Deming regression and linear transformation in a calibration cohort.[51] In this study, we used the following equation to harmonize partial volume-corrected mean FreeSurfer cortical SUVR measures between PiB and 18F-AV-45:

$$PiB\ SUVR = (18F{-}AV{-}45SUVR{-}0.0238)/0.7948$$

Transcriptomic and functional pathway analyses

Post-mortem brain sections were available from a subset of participants ($n = 96$) with preclinical AD ($n = 83$)[52,53] and healthy controls ($n = 13$) and evaluated by single-

nucleus RNA (snRNA) sequence analyses for differential VEC brain expression levels.[54,55] Functional pathway analyses for VEC in human brains were performed using STRING v11.5 for functional protein association networks (https://string-db.org).[56–58]

Statistical analyses

Analysis of variance, Student's t-tests, Fisher exact tests, or χ^2 tests were used to assess differences in demographic, cognitive, genotypic, CSF, or imaging biomarker variables between the clinical groups. Receiver Operating Characteristic (ROC) curve analyses assessed the ability of CSF biomarkers to predict brain amyloid positivity based on the CSF Aβ42/Aβ40 ratio or global amyloid burden on amyloid PET scans (IBM SPSS Statistics v28.0). Pearson correlations examined associations among CSF markers or between CSF markers and global amyloid burden on amyloid PET (IBM SPSS Statistics v28.0). Analyses were adjusted for multiple comparisons using the Bonferroni correction.

Linear regression analyses examined the associations of CSF or PET imaging markers with cognitive measures (PACC, FCSRT-Free, Digit-Symbol, Animal Fluency, and MMSE), adjusting for age, sex, education, *APOE4* genotype, and WMI. Mediation analyses were performed to determine whether CSF VEC levels mediated the effects of CSF Aβ42/Aβ40, global amyloid burden, or CSF p-tau181 on cognitive outcomes using the Sobel test[59] and bootstrapping methods (SPSSv28, Process v4.1 by Hayes).[60] CSF and imaging measures were all standardized to z scores prior to analyses. WMI measures were normalized by estimated total intracranial volumes prior to analyses. Statistical significance was defined as $p < 0.05$.

Results

Characteristics of the study cohort

This study included $n = 700$ cognitively normal (CDR 0) participants including $n = 558$ participants in the A+T ±N± cohort, $n = 259$ in the A+T+N± cohort, and $n = 106$ healthy biomarker-confirmed controls (A−T−N−). Participants in the A+T+N± cohort were older than those in the A+T±N± and control cohorts. Consistent with reports from similarly aged populations,[61] there were trends for a higher proportion of the *APOE4* genotype in cognitively normal participants on the AD pathological continuum (i.e., A+T±N± or A+T+N± cohorts) compared with controls. No significant differences in sex distributions or years of education were observed between the study cohorts. Demographic,

cognitive, genotypic, CSF, and imaging biomarker characteristics of the study cohort are summarized in Table 1.

CSF VEC levels are increased in preclinical AD

In our cohort, CSF VEC (mean relative fluorescence units [RFU] ± standard error [SE]) levels were significantly higher in individuals with A+T±N± (mean ± SE, 1593 ± 14, $p = 0.031$; $n = 558$) or A+T+N± (1671 ± 18, $p < 0.0001$; $n = 259$) classifications compared with biomarker-confirmed controls (A−T−N−; 1493 ± 31; $n = 106$). Participants in the A+T+N± cohort had significantly higher CSF VEC levels compared with those in the A+T±N± cohort ($p = 0.013$). Mean CSF VEC levels in each of the ATN classifications of our study cohort are shown in Figure S1A. Higher CSF VEC levels were observed in cognitively normal participants with progressively more advanced ATN classifications compared with controls.

We then evaluated the ability of CSF VEC levels to detect brain amyloid positivity in our combined CDR 0 cohort ($n = 700$) using the CSF Aβ42/Aβ40 ratio (a cutoff value of ≤0.10). The area under the curve (AUC) ± SE (95% confidence interval) for CSF VEC in detecting CSF amyloid positivity was 0.56 ± 0.03 (0.50–0.61, $p = 0.04$), while the AUC was 0.66 ± 0.02 (0.61–0.71, $p < 0.0001$) for CSF p-tau181, 0.64 ± 0.02 (0.59–0.69, $p < 0.0001$) for CSF tau, and 0.57 ± 0.03 (0.52–0.62, $p = 0.01$) for CSF NFL. We also examined the ability of CSF VEC to detect amyloid positivity based on the global amyloid burden in the subset of participants who underwent amyloid PET scans ($n = 534$). In these analyses, the AUC for the ability of CSF markers to detect amyloid PET positivity was 0.61 ± 0.03 (95% confidence interval, 0.55–0.67, $p = 0.001$) for VEC, 0.68 ± 0.03 (0.62–0.75, $p < 0.0001$) for Aβ42/Aβ40, 0.74 ± 0.03 (0.68–0.80, $p < 0.0001$) for p-tau181, 0.76 ± 0.03 (0.71–0.82, $p < 0.0001$) for tau, and 0.72 ± 0.03 (0.67–0.79, $p < 0.0001$) for NFL. The ROC curves for CSF markers in detecting amyloid positivity by CSF Aβ42/Aβ40 or amyloid PET scans are shown in Figure S1B and C.

Our results also suggest that combining CSF VEC with CSF Aβ42/Aβ40, p-tau181, or tau improved the ability of

Table 1. Demographic, cognitive, genotype, CSF, and imaging biomarker characteristics of the study cohort.

Characteristic	Controls ($n = 106$)	A+T±N± ($n = 558$)	A+T+N± ($n = 259$)	p value
Demographics				
Age (years), mean ± SE	66.7 ± 0.83	67.2 ± 0.40	70.2 ± 0.53	<0.0001*
Sex (n, % female)	51 (48%)	331 (59%)	154 (59%)	0.09
Education (years), mean ± SE	16.1 ± 0.24	15.9 ± 0.11	15.8 ± 0.16	0.48
APOE4 genotype + (n, %)[1]	29 (27%)	202 (36%)	106 (41%)	0.05
Cognitive measures[2]				
PACC, mean ± SE	0.05 ± 0.06	0.02 ± 0.03	−0.10 ± 0.05	0.04*
FCSRT-Free, mean ± SE	−0.007 ± 0.09	−0.007 ± 0.04	−0.16 ± 0.07	0.16
Digit-Symbol, mean ± SE	0.004 ± 0.11	0.005 ± 0.05	−0.10 ± 0.07	0.43
Animal fluency, mean ± SE	−0.005 ± 0.09	−0.009 ± 0.04	−0.20 ± 0.06	0.03*
MMSE, mean ± SE	0.06 ± 0.10	−0.009 ± 0.04	−0.05 ± 0.07	0.64
CSF biomarker measures				
VEC (RFU), mean ± SE	1493 ± 31	1593 ± 14	1671 ± 18	<0.0001*
Aβ42 (pg/mL), mean ± SE	1048 ± 23	683 ± 12	733 ± 20	<0.0001*
Aβ42/Aβ40, mean ± SE	0.13 ± 0.002	0.06 ± 0.001	0.05 ± 0.001	<0.0001*
p-tau181 (pg/mL), mean ± SE	38 ± 0.7	58 ± 1.2	80 ± 1.8	<0.0001*
tau (pg/mL), mean ± SE	195 ± 4.9	315 ± 8.0	446 ± 13	<0.0001*
NFL (pg/mL), mean ± SE	614 ± 24	775 ± 19	896 ± 29	<0.0001*
Imaging measures				
Mean cortical amyloid SUVR, mean ± SE	1.06 ± 0.01	1.44 ± 0.03	1.60 ± 0.05	<0.0001*
nWMI measures, mean ± SE[3]	4259 ± 425	3708 ± 184	4080 ± 318	0.36

APOE4, apolipoprotein E4; VEC, Vascular Endothelial-Cadherin (VE-cadherin); RFU, relative fluorescence units; Aβ, amyloid-peptide; p-tau181, tau phosphorylated at threonine 181; NFL, neurofilament light-chain; SE, standard error; PACC, Preclinical Alzheimer's Cognitive Composite; FCSRT-Free, free recall score from the Free and Cued Selective Reminding Test; Digit-Symbol, Digit-Symbol Substitution test; MMSE, Mini-Mental Status Examination; SUVR, standardized uptake value ratios; nWMI, normalized measures of white matter injury.
*Statistical significance was defined as $p < 0.05$.
[1] Individuals were considered *APOE4*+ if they had one or more copies of the *APOE4* allele.
[2] Data for all cognitive measures are presented as standardized z scores.
[3] WMI scores were normalized by FreeSurfer measures of intracranial volume.

these individual markers to predict amyloid PET positivity in our study cohort (CDR 0; $n = 534$). The AUC for the combinations of CSF VEC with each of CSF Aβ42/Aβ40, p-tau181, or tau in predicting amyloid PET positivity were 0.72 ± 0.03 (95% confidence interval, 0.66–0.78), 0.78 ± 0.02 (0.73–0.82), and 0.80 ± 0.02 (0.76–0.84), respectively ($p < 0.0001$) (Fig. S1C).

CSF VEC levels correlate with CSF and imaging AD biomarkers in preclinical AD

We examined correlations of CSF VEC levels with CSF (i.e., Aβ42/Aβ40) or imaging (i.e., global amyloid load on amyloid PET) markers of amyloid, tau (i.e., CSF p-tau181), and neurodegeneration (i.e., CSF tau and CSF NFL) in our study cohorts. In the CDR 0 cohort ($n = 700$), significant correlations were observed between CSF VEC and CSF p-tau181 ($r = 0.25$, $p < 0.0001$), tau ($r = 0.24$, $p < 0.0001$), NFL ($r = 0.39$, $p < 0.0001$), Aβ42/Aβ40 ($r = -0.10$, $p = 0.02$), and global amyloid burden ($r = 0.14$, $p = 0.001$). Similarly, in amyloid-positive individuals based on CSF biomarker cutoffs (i.e., A+T±N± cohort; $n = 558$), CSF VEC levels correlated with CSF p-tau181 ($r = 0.26$, $p < 0.0001$), tau ($r = 0.24$, $p < 0.0001$), NFL ($r = 0.29$, $p < 0.0001$), and Aβ42/Aβ40 ($r = -0.10$, $p = 0.04$) levels (Fig. S2). In the A+T+N± cohort ($n = 259$), significant correlations were also observed between CSF VEC levels and CSF p-tau181 ($r = 0.17$, $p = 0.005$), tau ($r = 0.16$, $p = 0.01$), NFL ($r = 0.25$, $p < 0.0001$), and Aβ42/Aβ40 ($r = -0.11$, $p = 0.04$) levels.

We then examined correlations of CSF VEC with CSF or imaging AD markers in the subset of individuals with significant amyloid deposition on amyloid PET scans (i.e., amyloid-PET positive individuals; $n = 112$). In this cohort, significant correlations were observed between CSF VEC and CSF p-tau181 ($r = 0.34$, $p = 0.0002$), tau ($r = 0.31$, $p = 0.0009$), NFL ($r = 0.50$, $p < 0.0001$), and global amyloid burden ($r = 0.27$, $p = 0.0037$) (Fig. 1).

CSF VEC levels correlate with cognitive impairment in preclinical AD

We first examined correlations between CSF biomarker measures and cognition using PACC and domain-specific cognitive scores in CSF Aβ + individuals (A+T±N±; $n = 558$), adjusting for age, sex, education, *APOE4* genotype, and WMI. Significant negative correlations were observed between CSF VEC levels and PACC scores ($r = -0.11$, $p = 0.01$), and individual cognitive scores for episodic memory (FCSRT-Free; $r = -0.14$, $p = 0.002$), language (animal fluency; $r = -0.10$, $p = 0.03$), and executive (Digit-Symbol; $r = -0.08$, $p = 0.04$) functions. Similarly, CSF Aβ42/Aβ40, p-tau181, tau, and NFL levels also

correlated with PACC, FCSRT-Free, animal fluency, and Digit-Symbol scores in this cohort. Conversely, MMSE scores were associated with CSF Aβ42/Aβ40, tau, and NFL, but not p-tau181 or VEC, levels in this cohort (Table 2).

We then examined correlations of CSF biomarkers with cognition in individuals with biomarker-confirmed AD (A+T+N±; $n = 259$) adjusting for age, sex, education, *APOE4* genotype, and WMI. In this cohort, significant correlations were observed between CSF VEC levels and PACC, FCSRT-Free, Digit-Symbol, and animal fluency scores. CSF Aβ42/Aβ40, p-tau181, tau, and NFL also correlated with PACC, FCSRT-Free, Digit-Symbol, and animal fluency scores in this cohort. Significant correlations were observed between MMSE scores and CSF Aβ42/Aβ40, but none of the other markers (VEC, p-tau181, tau, or NFL), in this cohort (Table 3). No significant correlations were observed between CSF biomarkers and cognition in the control group (A−T−N−, $n = 106$; data not shown).

We then examined associations of CSF VEC with cognition in the subset of individuals with significant amyloid PET deposition ($n = 112$). In this cohort, negative correlations were observed between CSF VEC and PACC ($r = -0.23$, $p = 0.01$), FCSRT-Free ($r = -0.22$, $p = 0.03$), Digit-Symbol ($r = -0.20$, $p = 0.04$), and to a lesser extent, animal fluency ($r = -0.16$, $p = 0.07$), after adjusting for covariates (age, sex, education, *APOE4* genotype, and WMI). Unadjusted correlations of standardized CSF VEC levels with global and domain-specific cognitive outcomes in this cohort are shown in Fig. 2.

CSF VEC mediates the relationship between amyloid or tau biomarkers and cognition in preclinical AD

We investigated whether CSF VEC, as a measure of endothelial injury, mediated the effects of amyloid and tau pathologies on cognitive outcomes in individuals with preclinical AD pathology. In CSF Aβ + individuals (A+T±N±; $n = 558$), CSF VEC was a significant mediator for CSF Aβ42/Aβ40 correlations with PACC (β estimate for indirect effect $= 0.019$, $p = 0.03$), FCSRT-Free ($\beta = 0.020$, $p = 0.04$), Digit-Symbol ($\beta = 0.015$, $p = 0.04$), and animal fluency ($\beta = 0.015$, $p = 0.04$). In this cohort, CSF VEC also significantly mediated effects of CSF p-tau181 on PACC ($\beta = -0.028$, $p = 0.001$), FCSRT-Free ($\beta = -0.023$, $p = 0.03$), Digit-Symbol ($\beta = -0.023$, $p = 0.02$), and animal fluency ($\beta = -0.026$, $p = 0.03$).

We then repeated these analyses in the subset of participants who underwent amyloid PET scans to evaluate whether CSF VEC mediated the relationship between
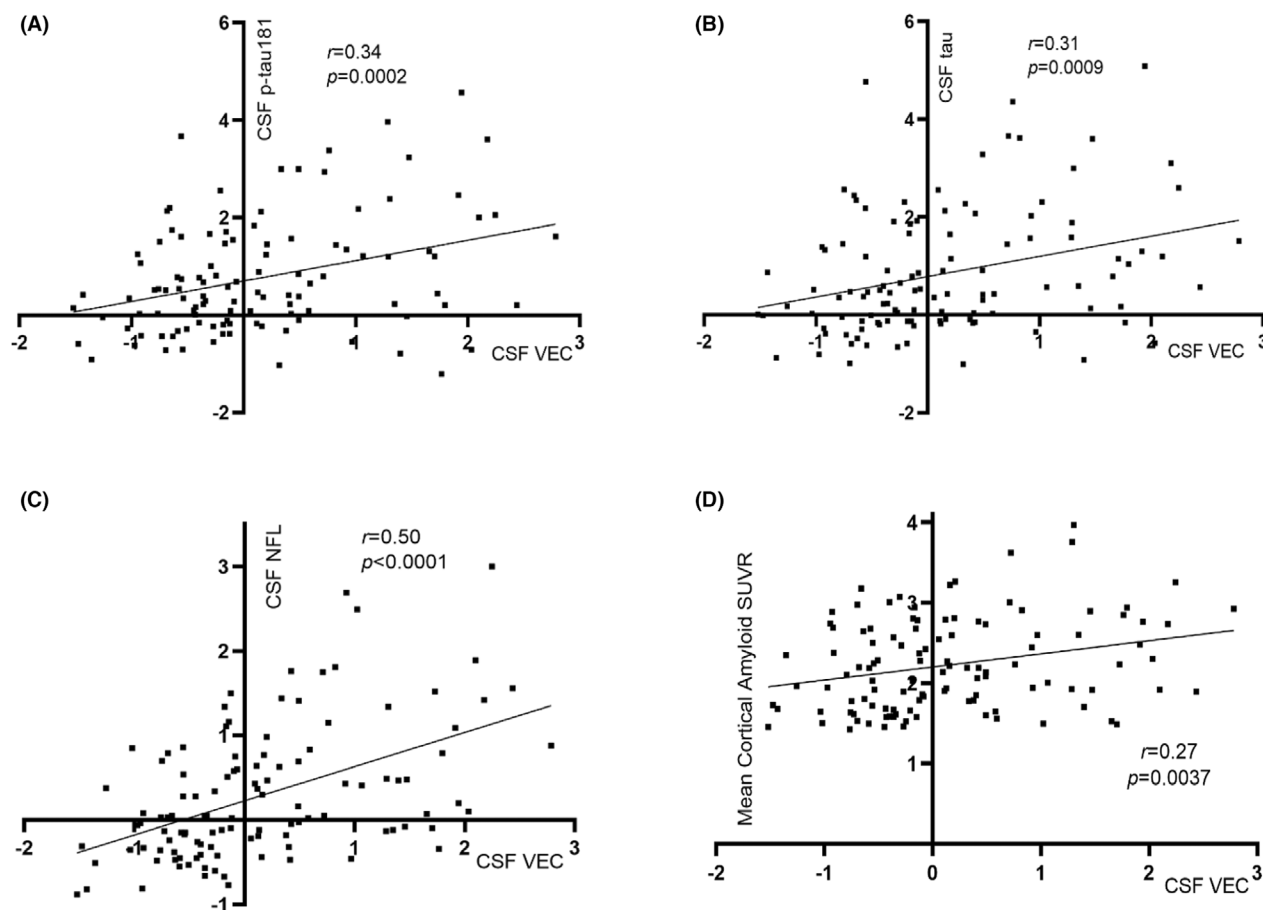
Figure 1. Correlations of CSF VEC with CSF and imaging biomarkers in preclinical AD. CSF VEC levels significantly correlated with (A) CSF p-tau181 ($r = 0.34$, $p = 0.0002$), (B) CSF tau ($r = 0.31$, $p = 0.0009$), (C) CSF NFL ($r = 0.50$, $p < 0.0001$), and (D) global amyloid burden measured as mean cortical amyloid SUVR ($r = 0.27$, $p = 0.0037$) in individuals with significant amyloid deposition on amyloid PET scan (i.e., amyloid-PET positive individuals; $n = 112$). CSF biomarker levels are shown as standardized z scores. VEC, VE-cadherin; p-tau181, tau phosphorylated at threonine 181; NFL, neurofilament light-chain; SUVR, standardized uptake value ratio.

global amyloid burden and cognition ($n = 534$). Consistent with our CSF biomarker data, global amyloid burden correlated with PACC ($r = -0.16$, $p = 0.0002$), FCSRT-Free ($r = -0.16$, $p = 0.0002$), Digit-Symbol ($r = -0.15$, $p = 0.003$), and animal fluency ($r = -0.10$, $p = 0.02$). CSF VEC mediated the relationship between global amyloid burden and PACC ($\beta = -0.034$, $p = 0.01$), FCSRT-Free ($\beta = -0.037$, $p = 0.02$), Digit-Symbol ($\beta = -0.031$, $p = 0.02$), and animal fluency ($\beta = -0.030$, $p = 0.04$) in this cohort.

Transcriptomic analyses of VEC expression in human AD brains

We analyzed brain snRNA-seq data from $n = 96$ cognitively normal participants in our cohort, including $n = 83$ with neuropathologically confirmed AD and $n = 13$ controls. Brain *CDH5* (i.e., VEC) was differentially expressed with lower expression in preclinical AD compared to controls (log 2-fold change, -0.41; $p = 0.02$).

Functional pathway analyses

Results of our functional pathway analyses suggest the presence of significant functional interactions between *CDH5* (i.e., VEC) and *APOE4* (Fig. 3). Specifically, VEC interacts with the vascular endothelial growth factor receptor-2 (*VEGFR2*; *KDR*), a cell surface receptor for *VEGF*, which plays an essential role in regulating angiogenesis, including endothelial cell growth, migration, and differentiation.[62] *VEGFA* forms a complex with neuropilin-1 (*NRP-1*) that binds to *VEGFR* on the endothelial cell surface.[63] Studies have shown that *APOE4* is associated with increased *NRP-1* expression resulting in

Table 2. Correlations between CSF biomarkers and cognitive measures in cognitively normal individuals with amyloid pathology (A+T±N±).

CSF biomarkers	Cognitive outcomes				
	FCSRT-Free	Animal fluency	Digit-Symbol	MMSE	PACC
VEC	−0.14 (0.002*)	−0.10 (0.028*)	−0.08 (0.043*)	−0.07 (0.13)	−0.11 (0.011*)
Aβ42/Aβ40	+0.15 (<0.001*)	+0.12 (0.007*)	+0.10 (0.035*)	+0.16 (<0.001*)	+0.19 (<0.001*)
p-tau181	−0.20 (<0.001*)	−0.14 (<0.001*)	−0.12 (0.010*)	−0.06 (0.17)	−0.20 (<0.001*)
tau	−0.23 (<0.001*)	−0.15 (<0.001*)	−0.12 (0.011*)	−0.10 (0.020*)	−0.22 (<0.001*)
NFL	−0.16 (<0.001*)	−0.11 (0.018*)	−0.12 (0.014*)	−0.11 (0.024*)	−0.17 (<0.001*)

Values represent β estimates; *p* values are shown in parenthesis. All CSF biomarker and cognitive measures were standardized to *z* scores prior to analyses. Analyses were adjusted for covariates (age, sex, education, *APOE4* genotype, and MRI measures of white matter injury [WMI]). This cohort consists of individuals with CDR 0 who meet the A+T±N± classification; *n* = 558.
*Statistical significance was defined as *p* < 0.05.
VEC, Vascular Endothelial-Cadherin (VE-cadherin); Aβ, amyloid-peptide; p-tau181, tau phosphorylated at threonine 181; NFL, neurofilament light-chain; FCSRT-Free, free recall score from the Free and Cued Selective Reminding Test; Digit-Symbol, Digit-Symbol Substitution test; MMSE, Mini-Mental Status Examination; PACC, Preclinical Alzheimer's Cognitive Composite.

Table 3. Correlations between CSF biomarkers and cognitive measures in cognitively normal individuals with biomarker-confirmed preclinical AD (A+T+N±).

CSF biomarkers	Cognitive outcomes				
	FCSRT-Free	Animal fluency	Digit-Symbol	MMSE	PACC
VEC	−0.13 (0.042*)	−0.12 (0.047*)	−0.14 (0.040*)	−0.07 (0.30)	−0.12 (0.041*)
Aβ42/Aβ40	+0.15 (0.022*)	+0.17 (0.009*)	+0.15 (0.036*)	+0.14 (0.03*)	+0.18 (0.002*)
p-tau181	−0.13 (0.035*)	−0.13 (0.045*)	−0.12 (0.052)	−0.09 (0.15)	−0.17 (0.003*)
tau	−0.11 (0.053)	−0.15 (0.018*)	−0.16 (0.02*)	−0.09 (0.16)	−0.16 (0.006*)
NFL	−0.13 (0.041*)	−0.15 (0.022*)	−0.23 (0.001*)	−0.09 (0.39)	−0.16 (0.005*)

Values represent β estimates; *p* values are shown in parenthesis. All CSF biomarker and cognitive measures were standardized to *z* scores prior to analyses. Analyses were adjusted for covariates (age, sex, education, *APOE4* genotype, and MRI measures of white matter injury [WMI]). This cohort consists of individuals with CDR 0 who meet the A$^+$T$^+$ N$^\pm$ classification; *n* = 259.
*Statistical significance was defined as *p* < 0.05.
VEC, Vascular Endothelial-Cadherin (VE-cadherin); Aβ, amyloid-peptide; p-tau181, tau phosphorylated at threonine 181; NFL, neurofilament light-chain; FCSRT-Free, free recall score from the Free and Cued Selective Reminding Test; MMSE, Mini-Mental Status Examination; Digit-Symbol, Digit-Symbol Substitution test; PACC, Preclinical Alzheimer's Cognitive Composite.

increased *VEGFR* signaling and overpermeabilization of existing vessels due to VEC phosphorylation and the subsequent loosening of endothelial tight junctions.[64]

Importantly, we have found significant interactions between *CDH5*, *NRP-1*, Plexin-A4 (*PLXNA4*), and semaphorin-3A (*SEMA3A*). Semaphorins have important functions in neurodevelopment, including guiding axonal growth, dendritic arborization, and regulating synaptic formation.[65] Activity-induced secretion of *SEMA3A* was found to mediate contextual memory formation by increasing AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor expression in hippocampal synapses via the *NRP-1–PLXNA4–SEMA3A* complex.[66] Together, our pathway analyses suggest that reduced VEC expression in AD brains is potentially associated with cognitive impairment through reduced *SEMA3A*-mediated hippocampal learning.

Discussion

Emerging evidence from animal and clinical studies suggests that endothelial injury is an early and prominent AD pathology, which is observed in most AD brains, including those in the earliest preclinical stages.[6–14] Structural changes consistent with endothelial dysfunction, such as increased pinocytic vesicles, laminin deposition in the basement membrane, collagen accumulation, and focal necrotic changes in AD occur independently of changes to other vascular constituents (smooth muscle and connective tissue), or other forms of cerebrovascular pathology (i.e., arteriolosclerosis, atherosclerosis, or cerebral amyloid angiopathy).[10–14] Transcriptomic studies suggest that endothelial pathways are among the most differentially expressed in human AD brains[67]; 30 out of 45 genes associated with AD are expressed in endothelial
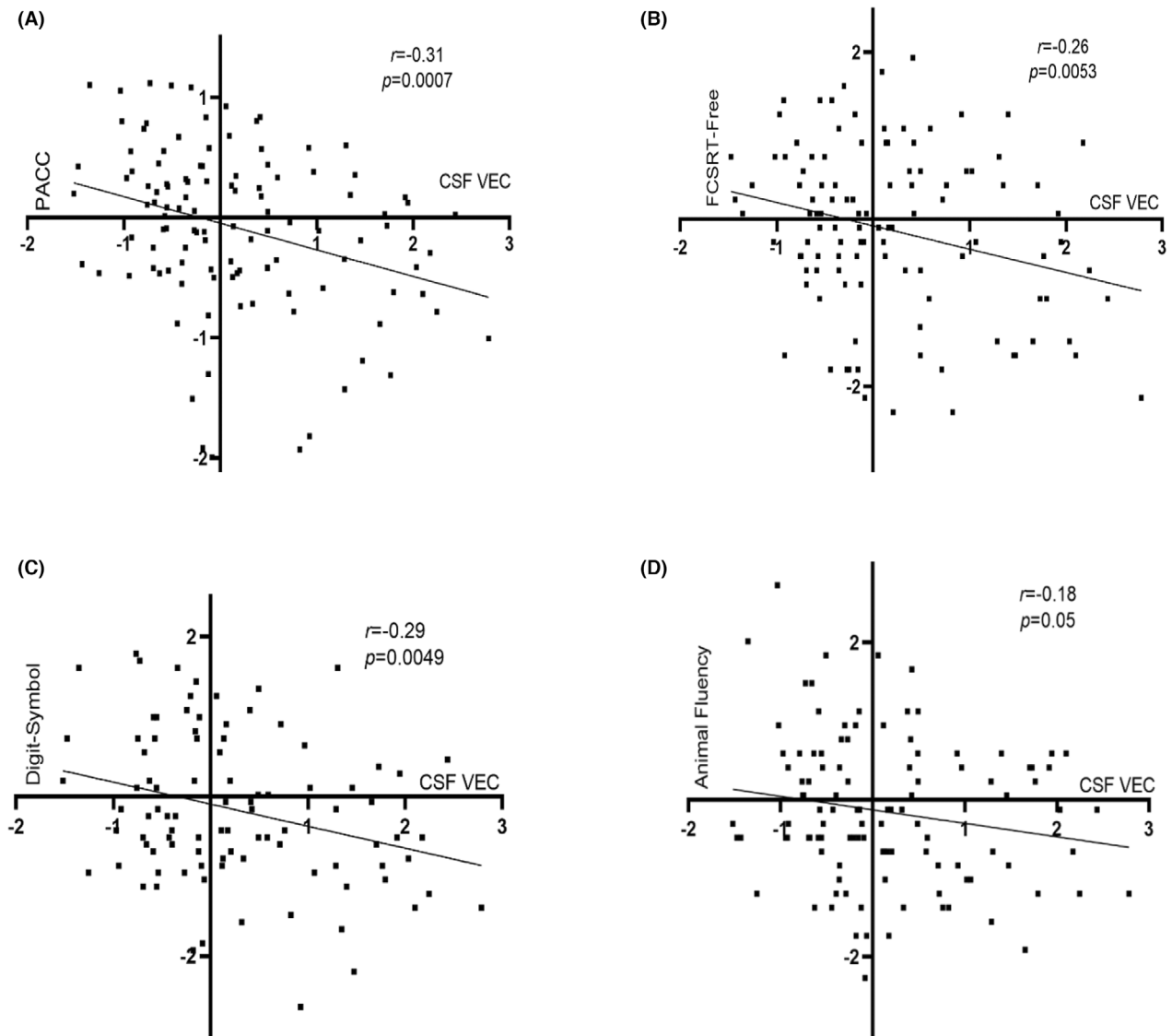
Figure 2. Correlations of CSF VEC with cognitive measures in preclinical AD. CSF VEC levels demonstrated significant negative correlations with (A) global cognition measured by PACC scores ($r = -0.31$, $p = 0.0007$; $n = 112$), (B) episodic memory measured by FCSRT-Free ($r = -0.26$, $p = 0.0053$; $n = 110$), (C) executive function measured by the Digit-Symbol Substitution test ($r = -0.29$, $p = 0.0049$; $n = 95$), and (D) language functions measured by Animal Fluency ($r = -0.18$, $p = 0.05$; $n = 111$) in the subset of individuals with significant amyloid deposition on amyloid PET scan (i.e., amyloid-PET-positive individuals; $n = 112$). Pearson correlation coefficient (r) values represent unadjusted correlations. CSF VEC levels and cognitive measures are shown as standardized z scores. VEC, VE-cadherin; PACC, Preclinical Alzheimer's Cognitive Composite; FCSRT-Free, free recall score from the Free and Cued Selective Reminding Test.

cells, and many of these genes have their highest expression levels in endothelial structures.[68]

Data from animal studies further support the notion that endothelial dysfunction is an early event in AD pathogenesis that may precede amyloid deposition[16] and cognitive deficits in AD transgenic or insulin-deficient mice.[17] Blocking leukocyte–endothelial interactions inhibits both Aβ deposition and tau hyperphosphorylation

and reduces memory loss in AD mouse models.[69] Furthermore, the current hypothetical model of temporal AD progression[3] has recently been challenged by multifactorial data-driven models[70] from longitudinal clinical cohorts which suggest that alterations to cerebrovascular structures are the earliest detectable changes in AD brains and may precede the first signs of amyloid or tau pathologies. Despite its importance, the role of brain
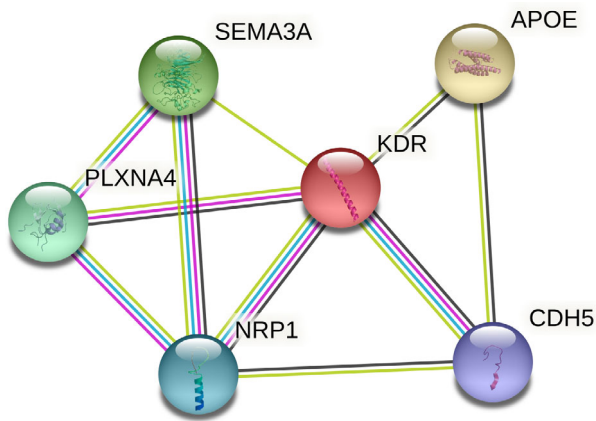
Figure 3. Functional pathway analyses of VEC in AD using STRING. Our functional pathway analyses suggest significant interactions of *CDH5* with *APOE* and the *NRP1-PLXNA4-SEMA3A* complex. The evidence score for *CDH5–APOE* interaction is 0.41 and that for the *CDH5–NRP-1* interaction is 0.51. Data-mining results suggest that *CDH5* functional interactions are predominantly with the *E4* allele of *APOE* (i.e., *APOE4*). Average node degree is 3.33 and average local clustering coefficient is 0.806. Network nodes represent proteins; splice isoforms or post-translational modifications are collapsed so that each node represents all the proteins produced by a single protein-coding gene locus. Edges represent protein–protein associations that are meant to be specific and meaningful. Color coding for the interactions are as follows: known interactions from curated databases (teal); known experimentally determined interactions (purple); predicted interactions from gene neighborhood (green), gene fusions (red), or gene co-occurrence (blue); predicted interactions from text-mining (yellow), co-expression (black), and protein homology (lavender). *APOE*, apolipoprotein E; *CDH5*, cadherin-5, aka VEC, vascular endothelial-cadherin; *NRP-1*, neuropilin-1; *PLXNA4*, Plexin A4; *SEMA3A*, semaphorin-3A; *KDR*, kinase insert domain receptor, aka vascular endothelial growth factor receptor 2, VEGFR2.

endothelial dysfunction in early AD pathogenesis has not been adequately investigated due to the absence of reliable endothelial biomarkers.

VEC (Cadherin-5; CD144) is an abundant calcium-dependent endothelium-specific adhesion molecule that is a key component of endothelial tight junctions[21] and is involved in regulating endothelial monolayer permeability, angiogenesis, and leukocyte adhesion.[21–23] Altered brain VEC expression levels have been reported in animal models of AD.[71] Consistent with these reports, we here demonstrate that brain VEC expression levels are reduced in human AD brains and are associated with increased CSF VEC levels, likely reflecting the release of abundant endothelial proteins into the extracellular space due to endothelial cell injury. To the best of our knowledge, our study is the first to examine CSF VEC levels in a large clinically and biologically well-characterized cohort of cognitively normal individuals, including those in the earliest preclinical stages of AD. We here show that CSF

VEC levels, reflective of endothelial injury, are increased in preclinical AD, correlate with biomarkers of amyloid, tau, and neurodegeneration, and are associated with cognitive outcomes including global cognition, episodic memory, executive, and language functions in preclinical AD. Our observations that CSF VEC levels in our cohort are closely associated with CSF markers of tau pathology and neurodegeneration suggest that AD pathologies beyond amyloid contribute to endothelial injury.[72] Importantly, our data suggest that endothelial injury partially mediates the effects of amyloid and tau on cognitive outcomes in preclinical AD.[72,73] Taken together, these findings support the notion that endothelial dysfunction, measured by CSF VEC, is an important pathological substrate of AD which contributes to cognitive impairment in even the earliest stages.

Proposed mechanisms by which endothelial dysfunction predisposes to neuronal or synaptic loss in AD include the impaired release of neurotrophic growth factors (e.g., VEGF and fibroblast growth factor [FGF]), disturbed neuronal glucose uptake due to reduced endothelial glucose GLUT1 transporters,[74] and reduced hippocampal expression of nitric oxide, an important mediator of synaptic transmission and long-term potentiation.[15,75] Data from our functional pathway analyses provide further mechanistic insight into the role of endothelial injury in mediating cognitive impairment and neurodegeneration in AD. Specifically, our analyses suggest that VEC interacts with NRP-1/PLXNA4, an important regulator of SEMA3A which guides axonal growth, synaptic plasticity, and neural circuit formation. Therefore, reduced VEC expression in AD brains is potentially associated with reduced axonal growth and synaptic plasticity due to impaired SEMA3A activity. These findings are consistent with evidence from other studies which suggests that endothelial dysfunction exerts direct effects on synaptic and neuronal functions that are independent of amyloid or tau.[75] Endothelial dysfunction is associated with reduced expression of presynaptic (e.g., synaptosomal-associated protein-25 [SNAP-25] and growth-associated protein-43 [GAP-43]) and postsynaptic (e.g., PSD95) proteins in human AD brains.[76–78] Furthermore, endothelial dysfunction displays regional and laminar patterns that parallel those of neuronal loss[8] and is preferentially localized in the vicinity of dystrophic neurites.[8,75]

The NIA-AA ATN Research Framework for AD[38] emphasizes the importance of identifying amyloid, tau, and neurodegeneration, using fluid or imaging biomarkers, to support a biological definition of AD independently of cognitive status. While this framework has allowed for a more accurate biological characterization of individuals in various stages of AD and improved

stratification of clinical trial cohorts, it does not include other important AD pathological substrates, such as endothelial dysfunction, which are known to contribute to neurodegeneration.[79] Therefore, in its current form, the ATN framework does not fully capture the molecular or pathological heterogeneity of AD, and there is a great need to identify markers that reflect AD pathologies beyond amyloid and tau and integrate these markers into the AD paradigm.[79] Our findings highlight several characteristics that support the potential value of CSF VEC as a marker of endothelial dysfunction in AD, including its abundant expression in brain endothelium,[21] well-established roles in mediating endothelial cell polarity and architecture,[21–23] significantly altered brain expression and CSF levels in AD compared to controls, the feasibility of accurate quantification of VEC levels in CSF, and its correlations with cognitive outcomes and other CSF or imaging markers of AD pathology.

The identification of novel biomarkers which can reliably detect and measure endothelial injury in AD has important clinical and research implications. Endothelial biomarkers will allow the integration of endothelial dysfunction into AD paradigms and provide a more accurate characterization of clinical and research cohorts (e.g., revising ATN to ATN-E in which "E" reflects endothelial injury).[79,80] Importantly, longitudinal AD studies which track endothelial injury, its association with clinical and radiological disease progression, and its relationship to other AD pathologies, will provide the opportunity to generate data-driven models that can accurately elucidate the temporal sequence of biomarker abnormalities and the relative contributions of different AD pathologies to neurodegeneration across different disease stages.[70,81] As endothelial injury significantly contributes to cognitive outcomes in preclinical AD, targeting endothelial injury in this critical time window offers a unique opportunity to delay symptom onset and disease progression. Therapeutic interventions which target endothelial dysfunction in other disorders may be repurposed to promote synaptic plasticity and axonal regrowth in AD.[82,83] Additionally, endothelial damage is a serious adverse event associated with investigational anti-amyloid therapies, including the recently FDA-approved agent, aducanumab.[84,85] Reliable endothelial biomarkers may provide a potentially useful tool to detect endothelial injury associated with these agents in the early stages and stratify individuals who may be at higher risk for such complications. Further studies are needed to investigate the role endothelial markers may play in clinical and research settings.

Our study has several strengths, including a large cohort ($n = 700$) with detailed cognitive, biomarker, and imaging data, the utilization of a highly reproducible assay for CSF VEC quantification, and the availability of neuropathological data from this cohort. However, our study is limited by the cross-sectional design and the availability of amyloid PET imaging data for only a subset of our cohort. It will be important to validate these findings in larger longitudinal cohorts of preclinical AD from different centers, using other proteomic platforms, and to examine the relationship between endothelial injury markers and clinical or radiological disease progression, including brain atrophy and amyloid or tau aggregation, over time. As emerging data from animal studies support a role for various vascular constituents (e.g., endothelium, pericytes, fibroblasts, extracellular matrix, basement membrane proteins) in AD pathogenesis,[86,87] future clinical studies which can reliably measure changes to each of these vascular constituents, using novel biomarkers, will provide further insight into vascular contributions to AD onset and progression.

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Conflict of Interest

CC has received research support from Biogen, Eisai, Alector, and Parabon. These funding agencies had no role in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. CC is a member of the advisory board of Vivid Genetics, Halia Therapeutics, and ADx Healthcare. JH is a paid consultant for Roche and Parabon Nanolabs. RT, RK, JS, and CLP have no conflict of interest.

Authors' Contributions

Rawan Tarawneh, MD: study design and conceptualization, contributed to data analyses and tools, performed study analyses and interpretation, and manuscript preparation and editing. Email: raltarawneh@salud.unm.edu. Rachel S. Kasper, MPH: data organization and biostatistical analyses and manuscript review and editing. Email: rkasper@salud.unm.edu. Jessie Sanford, MS: contributed to data analyses and tools including transcriptomic (RNA-seq) analyses, manuscript review and editing. Email: j.k.sanford@wustl.edu. Chia-Ling Phuah, MD, MMSc: contributed to data analyses and tools, imaging data analyses and interpretation, manuscript review and editing. Email: cphuah@wustl.edu. Jason Hassenstab, PhD: contributed to data analyses and tools, cognitive data interpretation, manuscript review and editing. Email: hassenstabj@wustl.edu. Carlos Cruchaga, PhD: study design, contributed to data analyses and tools, manuscript preparation and editing. Email: cruchagac@wustl.edu.

Data Availability Statement

Proteomic data from the Knight ADRC participants are available at the NIAGADS and can be accessed at https://www.niagads.org/knight-adrc-collection. Data was obtained from the Knight-ADRC through an approved data request. All data was de-identified.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Magnetic resonance imaging.
Figure S1. Diagnostic performance of CSF VEC levels in preclinical Alzheimer disease.
Figure S2. Correlations of CSF VEC with CSF AD biomarkers in cognitively normal individuals with amyloid pathology.