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Authors

Sapkota, Shraddha
Erickson, Kelsey
Fletcher, Evan
et al.

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Vascular Risk Predicts Plasma Amyloid β 42/40 Through Cerebral Amyloid Burden in Apolipoprotein E ε4 Carriers

Shraddha Sapkota, PhD; Kelsey Erickson, BS, BA; Evan Fletcher, PhD; Sarah E. Tomaszewski Farias, PhD; Lee-Way Jin, MD, PhD; Charles DeCarli, MD; for the Alzheimer's Disease Neuroimaging Initiative*

BACKGROUND: Understanding the neurobiological underpinnings between established multimodal dementia risk factors and noninvasive blood-based biomarkers may lead to greater precision and earlier identification of older adults at risk of accelerated decline and dementia. We examined whether key vascular and genetic risk impact the association between cerebral amyloid burden and plasma aβ (amyloid β) 42/40 in nondemented older adults.

METHODS: We used nondemented older adults from the UCD-ADRC (University of California, Davis-Alzheimer's Disease Research Center) study (n=96) and Alzheimer's Disease Neuroimaging Initiative (n=104). Alzheimer's Disease Neuroimaging Initiative was examined as confirmatory study cohort. We followed a cross-sectional design and examined linear regression followed by mediation analyses. Vascular risk score was obtained as the sum of hypertension, diabetes, hyperlipidemia, coronary artery disease, and cerebrovascular disease. *Apolipoprotein E* (*APOE*) ε4+ risk was genotyped, and plasma aβ42 and aβ40 were assayed. Cerebral amyloid burden was quantified using Florbetapir-PET scans. Baseline age was included as a covariate in all models.

RESULTS: Vascular risk significantly predicted cerebral amyloid burden in Alzheimer's Disease Neuroimaging Initiative but not in the UCD-ADRC cohort. Cerebral amyloid burden was associated with plasma aβ 42/40 in both cohorts. Higher vascular risk increased cerebral amyloid burden was indirectly associated with reduced plasma aβ 42/40 in Alzheimer's Disease Neuroimaging Initiative but not in UCD-ADRC cohort. However, when stratified by *APOE* ε4+ risk, we consistently observed this indirect relationship only in *APOE* ε4+ carriers across both cohorts.

CONCLUSIONS: Vascular risk is indirectly associated with the level of plasma aβ 42/40 via cerebral amyloid burden only in *APOE* ε4+ carriers. Nondemented older adults with genetic vulnerability to dementia and accelerated decline may benefit from careful monitoring of vascular risk factors directly associated with cerebral amyloid burden and indirectly with plasma aβ 42/40.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: Editorials ■ live birth ■ mitral valve insufficiency ■ mitral valve stenosis

A complex and dynamic multifactorial risk network has been linked with the highly heterogenous nature of Alzheimer disease (AD) and related dementias. Current biomarkers to measure hallmark characteristics of AD include invasive cerebrospinal fluid measures and expensive amyloid and tau positron emission

Correspondence to: Shraddha Sapkota, PhD, Department of Neurology University of California, 1590 Drew Ave, Unit No. 100, Davis, CA 95618. Email sapkota@ucdavis.edu
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Nonstandard Abbreviations and Acronyms

aβ	amyloid beta
AD	Alzheimer disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
BBB	blood-brain barrier
CSC	confirmatory study cohort
PET	positron emission topography
UCD-ADRC	University of California, Davis-Alzheimer's Disease Research Center

topography (PET) imaging.[1,2] Recent developments in advanced blood-based biomarker technologies have led to highly sensitive assays[3] to measure amyloid in blood decades before the onset of symptoms.[4] Understanding the neurobiological underpinnings of noninvasive and cost-effective blood-based biomarkers in relation to established amyloid PET markers will be fundamental to a broader application and earlier identification of cognitively unimpaired adults at high dementia risk and accelerated decline.

Ratio of amyloid β42 over amyloid β40 in plasma (plasma aβ [amyloid beta] 42/40) levels have been shown to accurately predict amyloidosis in nondemented older adult[5] brains and may be an early predictor of amyloidosis.[6] While a strong association has been established between amyloidosis and plasma aβ levels[7] with validation procedures underway,[8] a range of lifestyle factors and cardiovascular comorbidities also influence plasma.[7,9] Determining whether and how established modifiable risk factors impact the relationship between amyloid in the brain versus plasma will be necessary to understand their overall contribution to a larger heterogenous multifactorial risk network.[10]

One key modifiable risk domain that we examine in the present study is vascular risk. Vascular risk factors have been directly linked to cerebral amyloid burden in older adults,[11,12] and synergistic associations of vascular risk and amyloid burden has been associated with cognitive decline.[13] A range of inconsistent findings have been reported between vascular risk, amyloid burden, and plasma aβ 42/40 in the literature. For example, greater arterial stiffness was associated with greater amyloid burden after covarying for *Apolipoprotein E* (*APOE*) ε4+ risk and blood pressure.[14] *APOE* ε4 carriers with hypertension showed greater amyloid burden[15] whereas, higher blood pressure levels were linked to greater plasma aβ 40 levels in *APOE* ε4 noncarriers.[16] The impact of vascular risk factors on amyloid deposition, therefore, may be exacerbated by genetic vulnerability. *APOE* ε4 genetic risk has consistently been identified with amyloid burden and accelerated decline in dementia and has also been shown to play an important moderating role in key associations between risk markers and cognitive decline.[17] *APOE* allelic risk and plasma aβ 42 may be combined to predict amyloid PET positivity in older adults at high dementia risk.[18]

In the present study, we took a multifactorial approach by examining how vascular and genetic risk work together to impact the validated association between amyloid burden and plasma aβ 42/40. We used an ethnoracially diverse cohort[19] to test our research aims. First, we confirmed the association between amyloid burden and plasma aβ 42/40 in our diverse sample. Second, we investigated whether vascular risk predicts (1) plasma aβ 42/40, (2) cerebral amyloid burden, and (3) plasma aβ 42/40 through cerebral amyloid burden (indirect association). We hypothesized that higher vascular risk score would independently predict higher cerebral amyloid burden and lower plasma aβ 42/40 levels. Third, we tested an effect modification of *APOE* ε4-/ε4+ on the indirect association of vascular risk on plasma aβ 42/40 through cerebral amyloid burden. We hypothesized that higher vascular risk indirectly predicts plasma aβ 42/40 levels through cerebral amyloid burden and this association will be stronger in *APOE* ε4 carriers. Figure 1 displays the sequential steps to test this potential multimodal risk association. We aimed to validate our findings using the AD Neuroimaging Initiative (ADNI) data as a confirmatory study cohort (CSC).

METHODS

Data Availability Statement

Subject to IRB limitations, we share all data collected by the ADRC. In accordance with NIH policy on data sharing, we reserve the right of initial publication related to components of our data that are central to our research program for 2 years.

Participants

We used data from the UCD-ADRC (University of California, Davis-Alzheimer's Disease Research Cohort),[19] an ethnoracially diverse longitudinal study representing community dwelling older adults who are cognitively normal or diagnosed as mild cognitive impairment or demented. The UCD-ADRC and all data collection are in full and certified compliance with the human/institutional review board. Written informed consent was obtained from all participants or their legal representatives. UCD-ADRC data collection started from 2001 and is ongoing. Vascular risk factor and DNA data was collected at baseline. Cerebral amyloid burden data were collected between 2011 and 2018 and plasma aβ 42/40 between 2002 and 2019. Ethnicity and racial status were self-reported, and standard criteria and methods were followed for cognitive status diagnosis.[20] Participants received a multidisciplinary clinical evaluation using the Uniform Data Set battery.[20,21] For the present study, we used a subsample of cognitively normal participants with amyloid PET and plasma data (*N*=96; mean age=72.97 [6.14] years old, *n* women=60; see the Table; Figure 2). This manuscript follows the STROBE reporting guideline.[22]

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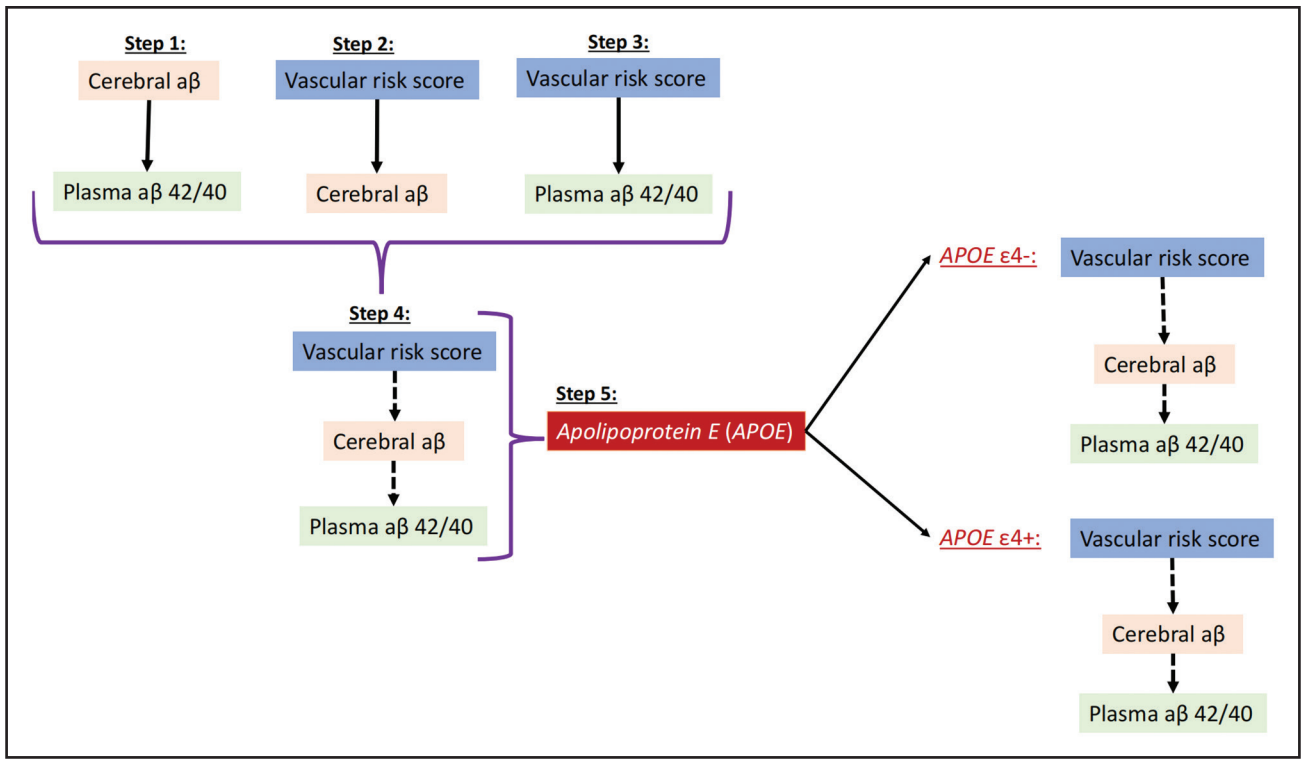

Figure 1. Role of vascular, genetic, and cerebral amyloid burden on plasma aβ (amyloid β) 42/40: sequential steps to test a potential multifactorial risk network associated with noninvasive biomarker in nondemented older adults.

CSC data were obtained from the ADNI database (adni.loni.usc.edu). ADNI was launched in 2003 as a public-private partnership, led by principal investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of mild cognitive impairment and early AD. For up-to-date information, see www.adni-info.org. We included *n*=104 nondemented older adults (mean age=73.59 (6.24) years; *n* women=56) from ADNI-2 recruited between 2011 and 2012 and ADNI-3 recruited between 2017 and 2021. Data collection for variables for interest was different. Specifically, nondemented (including 74 CN and 30 subjective memory complaints; see the Table; Figure 2) participants with clinical, PET imaging, and *APOE* genotype data in the "ADNIMERGE" table and plasma data from "BATEMAN LAB" table downloaded on April 19, 2022, were included. Vascular risk factor scores were derived using risk factors in the Medication History and the Modified Hachinski tables.

Plasma aβ 42/40 Collection

UCD-ADRC blood samples were randomly obtained, processed within 2 hours, and stored in 250 µL aliquots at −80 °C using previously reported standard procedures.[23] ADNI plasma samples were assayed in the Biomarker core laboratory using Innogenetics research on a Luminex immunoassay platform.[24] Additional methodological details for plasma aβ are provided in the Supplemental Methods for the UCD-ADRC and on adni.loni.usc.edu for ADNI.

PET

Image Acquisition

In the UCD-ADRC, florbetapir-PET scans were acquired on a Siemens Biograph mCT 40 PET machine during a 50-to-70-minute interval following a 10 mCI (370 MBq) bolus injection of florbetapir (^{18}F).[25] In ADNI, scans were acquired using ^{18}F-florbetapir PET with 4×5-minute frames acquired at 50 to 70 minutes after injection.[26] PET analysis details are provided in the Supplemental Methods.

Genotyping

APOE genotyping for rs7412 and rs429358 were assayed from DNA by the National Centralized Repository for Alzheimer's Disease and Related Dementias[27] in both the UCD-ADRC and ADNI. All ε2/ε4 case were excluded in the ADRC because of conflicting reports on ε2 protective effects versus ε4 risk associations,[28] and *APOE* genotyping in "ADNI merge table" was used for ε4−/ε4+ groupings. *APOE* ε4+ carriers were coded as 1 and nonrisk carriers (*APOE* ε4−) as 0.

Vascular Risk

Vascular risk score was obtained using 5 vascular risk factors in the UCD and ADNI. The presence (score of 1) or absence (score of 0) of hypertension, diabetes, hyperlipidemia, coronary artery disease, and cerebrovascular disease was used in UCD-ADRC.[29] The presence or absence of hypertension, diabetes, stroke, smoking, and cardiovascular disease[30] was used in ADNI. Higher score indicated greater severity (maximum score of 5). This vascular risk score obtained from categorical variables was then transformed

Table. Characteristic of Study Participants in the UCD-ADRC and ADNI by *Apolipoprotein E* (*APOE*) ε4+ Status

	UCD-ADRC				ADNI			
	Total	*APOE* ε4−	*APOE* ε4+	*P* value	Total	*APOE* ε4−	*APOE* ε4+	*P* value
N	96	61	33	…	104	71	33	…
Age, y	72.97 (6.14)	73.61 (6.22)	71.76 (6.09)	0.169	73.59 (6.24)	74.20 (5.75)	72.27 (7.08)	0.142
Sex (M/W)	36/60	20/41	16/17	0.138	48/56	61/61	41/33	0.175
Education, y	14.68 (3.94)	14.20 (4.23)	15.61 (3.33)	0.101	16.66 (2.51)	16.48 (2.62)	17.06 (2.25)	0.274
Ethnicity (Black/Hispanic/White)	21/25/43	9/20/26	10/5/17	0.363	…	…	…	…
Diagnosis (normal/SMC)	96/0	61/0	33/0	…	74/30	53/17	21/13	…
Plasma aβ 42/40	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.551	0.12 (0.01)	0.12 (0.01)	0.12 (0.01)	0.475
Cerebral amyloid (SUVR)	1.12 (0.18)	1.08 (0.16)	1.18 (0.17)	0.007	1.73 (0.20)	1.11 (0.16)	1.28 (0.24)	<0.001
Vascular risk score	0.54	0.56 (0.38)	0.49 (0.31)	0.407	2.06 (1.20)	1.93 (1.16)	2.33 (1.24)	0.110

Means and SDs are in brackets. aβ indicates amyloid β; ADNI, Alzheimer's Disease Neuroimaging Initiative; SMC, Subjective Memory Complaint; SUVR, standardized uptake value ratio; and UCD-ADRC, University of California, Davis-Alzheimer's Disease Research Center.

into a percentage representing a continuous score of 0-1 in the UCD to account for missing values on 1 or more of the vascular risk factor questions. The average across nonmissing data points was taken and divided by the number of nonmissing data points. The 0 to 1 scale is a percent of nonmissing items that shows positive symptoms/signs. The UCD-ADRC vascular risk score was recently developed to use across studies in the cohort and the ADNI vascular risk score was composed specifically for the present study. With no missing items in the ADNI vascular risk score calculation, we did not transform the ADNI vascular risk scores. All vascular risk factor information was obtained from each participant's medical history and records, and any medications used at the initial evaluation in clinic.

Statistical Analysis

Baseline participant characteristics were compared by *APOE* ε4 genotype using analysis of variance for continuous variables and a chi-squared test for categorical variables. Continuous measures were summarized using means and standard deviations whereas categorical measures were summarized using counts and percentages.

Regression analyses were used to test all 3 direct associations in Mplus Version 8.6.[31] All dependent missing values were assumed to be missing at random and were estimated using maximum likelihood. Cases with missing predictor values were removed using list-wise deletion in Mplus. In the UCD-ADRC, we had missing data for vascular risk (n=1), plasma aβ 42/40 (n=1), and *APOE* (n=2). We did not have

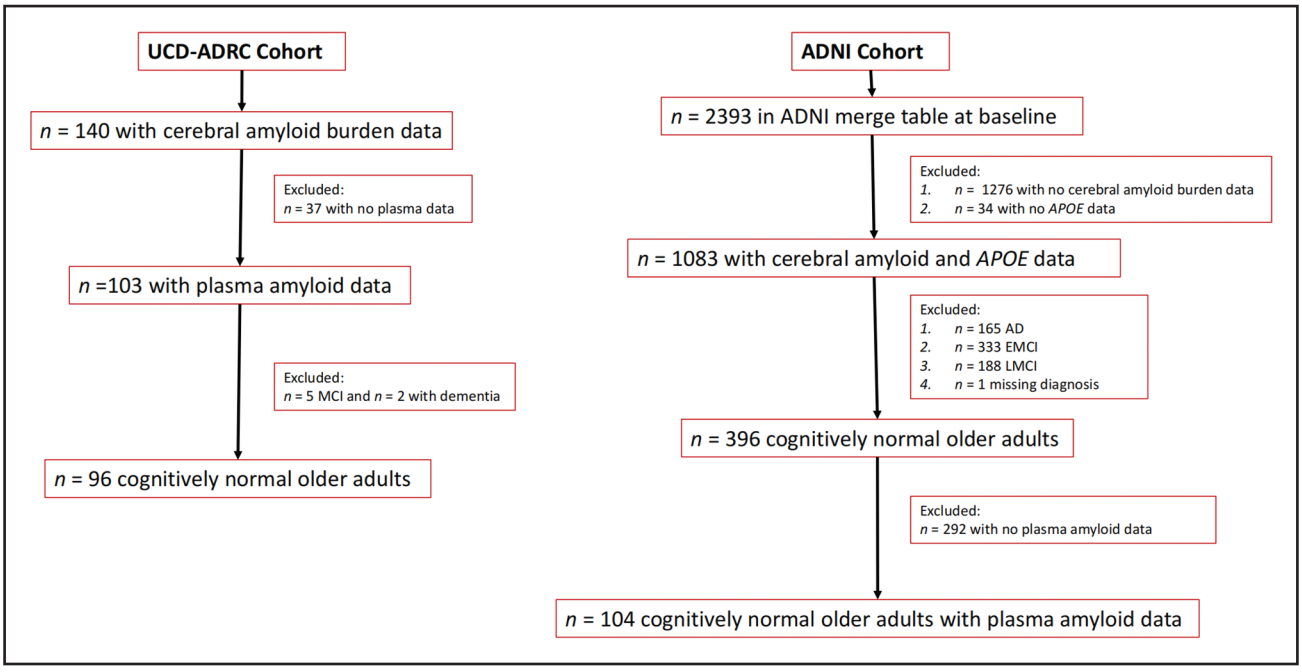

Figure 2. Flow diagram of study inclusion and exclusion criteria in the University of California-Alzheimer's Disease Research Center (UCD-ADRC) and Alzheimer's Disease Neuroimaging Initiative (ADNI).

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any missing data for variables of interest in ADNI. First, plasma aβ 42/40 was regressed on cerebral amyloid PET. Second, plasma aβ 42/40 was regressed on vascular risk score. Third, cerebral amyloid PET was regressed on vascular risk score. Indirect association between vascular risk and plasma aβ 42/40 through cerebral amyloid burden was tested with mediation model which calculates the indirect effect with bias-corrected bootstrapped 95% CIs (using the Baron-Kenny method), where the dataset is resampled with replacement over 5000 iterations.[32,33] We further tested this as stratified *APOE* ε4-/ε4+ groups. Baseline age was included as covariate in all models. All models were identified with significant indirect effect when the association between vascular risk score and plasma aβ 42/40 through cerebral amyloid burden was significant.

RESULTS

Demographics

In the UCD-ADRC, mean baseline age of participants was 72.97 (6.14) years with 62.5% women and average education of 14.68 (3.94) years. Cerebral amyloid levels were significantly lower in *APOE* ε4− (*n*=61) group compared with *APOE* ε4+ (*n*=33) groups. In ADNI, mean baseline age was 73.59 (6.24) years with 54% women and average education of 16.66 (2.51) years. Cerebral amyloid levels were significantly lower in *APOE* ε4− (*n*=71) group compared with *APOE* ε4+ (*n*=33) groups. Descriptive statistics for all characteristics and comparisons by *APOE* ε4+ are presented in the Table.

Direct Associations Between Vascular Risk, Amyloid Burden, and Plasma aβ 42/40

We confirmed that cerebral amyloid burden was associated with plasma aβ 42/40 in both the UCD-ADRC and the ADNI CSC (Figure 3). As expected, higher cerebral amyloid burden was associated with lower plasma aβ 42/40 (UCD-ADRC:β=−0.012, SE=0.006; *P*=0.039; ADNI:β=−0.015, SE=0.006; *P*=0.013). Higher vascular risk score predicted greater amyloid burden in ADNI (β=0.043, SE=0.016; *P*=0.006) but not in the UCD-ADRC cohort (β=0.096, SE=0.050; *P*=0.053). Vascular risk was inconsistently associated with plasma amyloid aβ 42/40 across the 2 cohorts. Specifically, higher vascular risk was associated with higher plasma amyloid aβ 42/40 levels (opposite direction than expected) in UCD-ADRC (β=0.008, SE=0.003; *P*=0.007) but not in ADNI (β=−0.001, SE=0.000; *P*=0.416).

Indirect Association of Vascular Risk and Plasma aβ 42/40 Through Cerebral Amyloid Burden and as Stratified by *APOE* ε4+ Risk

Inconsistent associations were observed between the 2 cohorts for the indirect association of vascular risk on plasma amyloid aβ 42/40 levels through cerebral amyloid burden. Specifically, higher vascular risk indirectly predicted lower plasma amyloid aβ 42/40 levels in the whole group in ADNI but not in the UCD-ADRC cohort (UCD-ADRC:β=−0.001 [90% CI, −0.003 to 0.000], SE=0.001; *P*=0.078; ADNI:β=−0.001 [90% CI, −0.002 to 0.000], SE=0.000; *P*=0.006). However, when this was stratified by *APOE* ε4+ risk, we observed significant indirect association in both cohorts for *APOE* ε4 carriers (UCD-ADRC:β=−0.004 [90% CI, −0.009 to −0.002), SE=0.002; *P*=0.030; ADNI: β=−0.002 [90% CI, −0.004 to −0.001], SE=0.001; *P*=0.027) but not in the *APOE* ε4− group (UCD-ADRC:β=−0.001 [90% CI, −0.002 to 0.000], SE=0.001; *P*=0.474; ADNI:β=0.000 [90% CI, −0.001 to 0.000], SE=0.000; *P*=0.515).

DISCUSSION

In this cross-sectional study, higher vascular risk score indirectly predicted lower plasma aβ 42/40 through

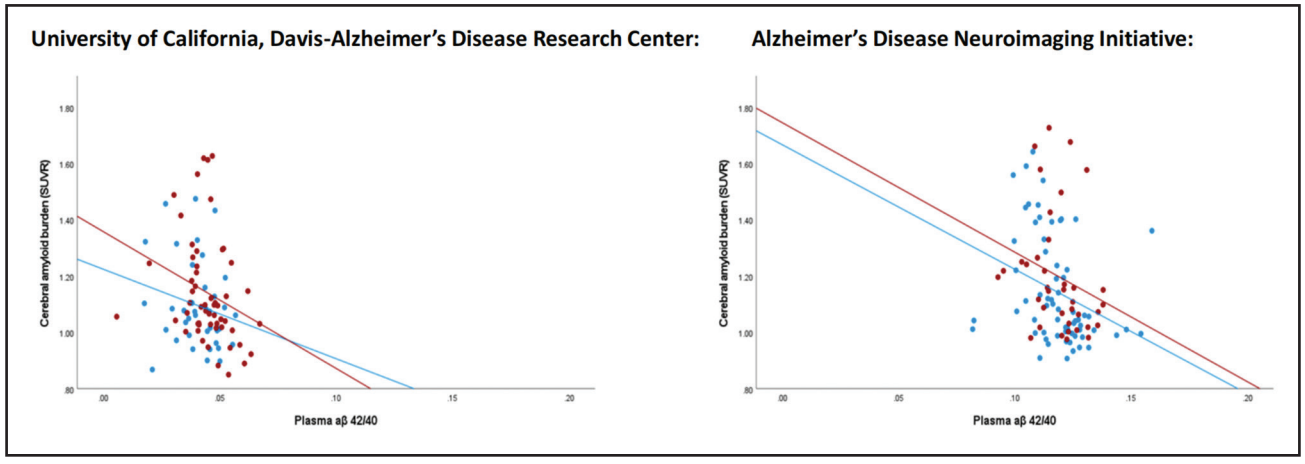

Figure 3. Lower plasma aβ (amyloid β) 42/40 was associated with higher cerebral amyloid burden across low and high vascular risk score groups (split at the mean; low vascular risk=blue, high vascular risk=red) in both cohorts.

cerebral amyloid burden only in *APOE* ε4 carriers. We validated our indirect association in *APOE* ε4 carriers using a CSC. Our findings suggest an indirect relationship of vascular risk factors on a key blood-based biomarker (aβ 42/40) through amyloid burden in nondemented older adults with high genetic susceptibility (*APOE* ε4 carriers).

Elevated cerebral amyloid burden has consistently been associated with lower plasma aβ 42/40.[7] We confirm this fundamental association in both our cohorts and add to the increasing literature aimed at understanding the underlying mechanism between cerebral amyloid burden and plasma amyloid levels.[34] We note that plasma aβ 42/40 was lower in the UCD-ADRC versus the ADNI cohort, but the relationship between plasma amyloid and amyloid burden in the brain was observed in both groups. Our main goal, which was to understand how this association changes when we account for vascular risk and *APOE* risk. Regardless of highly different demographics and smaller sample size, the association between amyloid in the brain and plasma is influenced by overall vascular risk score, and this is only consistently observed in *APOE* ε4 risk carriers. While our findings were based on cross-sectional data, future work will benefit from examining this association over time[35] with larger sample sizes to fully capture the predictivity of plasma aβ 42/40 and amyloidosis in relation to neurodegeneration, disease pathology, phosphorylated tau, and cognitive decline.

Higher vascular risk contributed to greater cerebral amyloid burden but was inconsistently associated with plasma aβ 42/40. Vascular risk may contribute to AD pathologies by influencing aβ production/clearance.[36] Specifically, cholesterol and high blood pressure is associated with greater amyloid burden.[37] Elevated blood pressure may compromise vascular integrity leading to cerebral amyloid angiopathy and impaired aβ clearance from the brain.[38] Autopsy findings show that amyloid in combination with high blood pressure may deteriorate vessel walls and reduce clearance.[38] Arterial hypertension mouse models show that chronic hypertension may increase blood-brain barrier (BBB) permeability and lead to greater amyloid deposition in plasma. Thus, only when vascular risk is examined in conjunction with amyloid burden, the levels of plasma aβ 42/40 may be justified.

Inconsistent reports on vascular risk and AD pathology (ie, amyloid and tau burden) in nondemented older adults highlight the need for more in-depth research and replication in this area.[11–13] With smaller sample size, we did not account for moderating effects of vascular medication use,[12] but these should be considered in future work. Cerebral aβ tends to increase before reaching a peak and then declining just before the onset of clinical symptoms which may also contribute towards inconsistent findings between cerebral aβ and plasma aβ.[39] We included nondemented older adults in our sample who may not have high amyloid burden and may not have reached their peak before the onset of clinical symptoms. Thus, longitudinal follow-up would help clarify the inconsistent findings for vascular risk factors and plasma aβ and further confirm the indirect association of vascular risk on plasma aβ through cerebral amyloid burden. Although we did not examine this in the present study, vascular association between amyloid and plasma aβ 42/40 should be tested in relation to tau deposition, the other major characteristic associated with AD.[34]

Nondemented older adults with both high vascular risk score and *APOE* ε4+ risk may be at a higher risk for amyloidosis. Our indirect findings suggest that vascular risk does not directly influence plasma aβ 42/40 but should be examined in conjunction with amyloid burden to fully capture the impact of vascular and genetic risk. Specific vascular risk factors such as hypertension and cholesterol may change the impact of APOE (a major cholesterol transporter in the brain) linked to cholesterol metabolism.[40] APOE ε4 isoform increases plasma low density lipoproteins suggesting that *APOE* ε4 carriers have increased cholesterol and vascular risk.[41] This leads to a higher aβ binding affinity, which may indirectly contribute toward vascular and AD pathologies.[42] *APOE* is also known to have pleiotropic effect whereby ε4 carriers are at higher risk of cognitive decline and dementia in old age but show better performance and excellence in younger adults.[43] *APOE* ε4 carriers show high amyloid burden than the ε4− group,[44] and this is also observed in autopsy findings for nondemented older adults.[45] APOE protein is highly involved in maintaining BBB integrity, thus a direct effect of amyloid clearance from the brain into plasma.[46] The integrity and permeability of BBB have been examined in mice,[47] where *APOE* ε4 carriers show greater permeability and increased transport across the BBB versus *APOE* ε3 groups leading to lower aβ clearance and impaired BBB directly influencing the amount of plasma aβ.[48]

Our study had strengths as well as limitations. A first strength is that we validated our main findings from an ethnoracially diverse cohort (composed of Blacks, Hispanics, and Whites) through community recruitment to a large longitudinal observational study composed of mainly Whites recruited across the United States and Canada. We confirmed our findings even in highly differing populations. Second, by identifying how plasma amyloid is associated with other established non-modifiable and modifiable dementia markers, we contribute to the large literature and potential clinical application of blood-based biomarkers to detect high risk individuals.

Regarding limitations, first, we had a relatively small sample size (n=96) to test a complex multimodal risk

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association. However, we used a CSC (n=104) to confirm our findings and shortcomings associated with small sample size. Second, vascular risk factors in the risk score were slightly different between the 2 cohorts which may have resulted in only borderline significance in the UCD-ADRC. Third, we did not examine longitudinal associations and the absence of a longitudinal time order limits our mediation results in a cross-sectional alone. This should be the next step to test how these synergistic associations change with time and in other subpopulations including dementia groups. Fourth, although we examined both amyloid PET and plasma aβ 42/40 in nondemented older adults, future work should consider including cerebrospinal fluid measurements to determine the role of cerebrospinal fluid amyloid levels and neurodegeneration (linked to vascular risk, *APOE*, and amyloidosis) in this complex and dynamic network across the dementia continuum. Fifth, with a smaller sample size we did not test for any sex differences in plasma aβ 42/40 and should also be considered in future work.

CONCLUSIONS

In conclusion, our findings suggest that monitoring and enhanced management of vascular risk factors in nondemented older adults may provide adults with *APOE* ε4+ risk an added layer of protection associated with high cerebral amyloid burden and lower plasma aβ 42/40. Vascular integrity in combination with genetic vulnerability are key components of preclinical trajectories. Thus, applying a multifaceted approach[49] with established (cerebral aβ) and noninvasive (plasma aβ) biomarkers for earlier detection of older adults at high risk and personalized intervention programs will be key to offsetting projected increases[50] in dementia incidence and prevalence.

ARTICLE INFORMATION

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Affiliations

Department of Neurology (S.S., E.F., S.E.T.F., C.D.), University of California, and Department of Pathology and Laboratory Medicine (K.E., L.-W.J.), University of California, Davis.

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Disclosures

None.

Supplemental Material

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