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RESEARCH ARTICLE

MRI free water mediates the association between diffusion tensor image analysis along the perivascular space and executive function in four independent middle to aged cohorts

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Abstract

INTRODUCTION: Diffusion tensor image analysis along the perivascular space (DTI-ALPS) index was proposed for assessing glymphatic clearance function. This study

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evaluated DTI-ALPS as a biomarker for cerebral small vessel disease (cSVD) related vascular cognitive impairment and dementia (VCID).

METHODS: Four independent cohorts were examined. A composite score of executive function (UDS3-EF) was used to evaluate EF status. The association between the ALPS index and UDS3-EF scores and the mediator effect of free water in white matter (WM-FW) on such association was analyzed.

RESULTS: The ALPS index was significantly associated with UDS3-EF scores in all cohorts. Additionally, WM-FW mediates the relationship between the ALPS index and UDS3-EF scores.

DISCUSSION: Lower ALPS index may be a surrogate marker of glymphatic dysfunction, which is associated with impaired EF, and this association is mediated by the interstitial fluid (ISF) drainage ISF in WM, providing a clinical rationale for using ALPS index as a biomarker for cSVD-related VCID.

KEYWORDS
cerebral small vessel disease (cSVD), diffusion tensor image analysis along the perivascular space index (DTI-ALPS), free water (FW), glymphatic system (GS), vascular cognitive impairment and dementia (VCID)

Highlights

- This is the first study to investigate the mediation role of interstitial FW fraction (WM-FW) on the relationship between glymphatic clearance (ALPS index) and EF (UDS3-EF scores) in four independent middle to aged cohorts at risk for cSVD.
- This study identified that ALPS index was independently associated with UDS3-EF scores after adjusting for demographics, VRFs, and WM hyperintensity burden and that WM-FW mediated this association in all middle to aged cohorts.
- Our findings suggest that in middle to aged individuals, glymphatic dysfunction (reflected by ALPS index) is strongly associated with EF and that this association is mediated by the ISF drainage in WM.
- This study provides a strong clinical rationale for the use of the ALPS index as a marker of cognitive function in multi-site observational studies and clinical trials to monitor and prevent VCID.

1 | BACKGROUND

Emerging data have shown that cerebral small vessel disease (cSVD) was the most common cause of vascular cognitive impairment and dementia (VCID).[1–3] VCID is estimated to be the second most common type of dementia after Alzheimer's disease (AD), with major deficits in executive function (EF), attention, memory, language, and information processing, that can seriously affect the activities of daily living of patients.[4] As with AD, the incident and severity of VCID are recognized as an urgent public health crisis.[1] Several mechanisms have been proposed to link cSVD to cognitive impairment, including blood–brain barrier (BBB) disruption, cerebral blood flow decreases, vascular gray matter and white matter (WM) injuries, oxidative stress, and inflammation.[3] The glymphatic system (GS) is a discovered putative clearance pathway within the central nervous system (CNS), which facilitates the clearance of the waste solute from the brain via the interstitial fluid (ISF)-cerebrospinal fluid (CSF) exchange, maintaining cerebral homeostasis.[5] GS dysfunction was thought to be involved in the pathophysiology and probable etiology of cSVD as well as VCID.[6–8] However, how changes in GS affect the cognitive function in cSVD is still poorly understood.

The diffusion tensor image analysis along the perivascular space (DTI-ALPS) is an approach to calculate the differences of the water diffusivity in the WM fiber tracts (projection and association fibers) between that in the direction of parallel and perpendicular to the perivascular space (PVS) around the medullary veins at the level of lateral ventricle body.[9] This approach hypothesizes that histological changes in the projection and association fibers may be attributable

to pathology involving the PVS.[9] Due to its noninvasive and rather simple nature, DTI-ALPS approach is currently gaining growing attention and has expanded its application in a variety of diseases and conditions.[9] It has shown favorable cross-vendor test–retest repeatability in a number of large sample cohorts.[10–12] Lower DTI-ALPS index indicates that free water (FW) movement in the PVS direction is not dominant, which has been reported to be associated with impairment of glymphatic clearance function in cSVD patients[13,14] and shown to be independently related to VCID.[7,15,16] Another DTI-based FW index was recently proposed to enable the extraction of the extracellular water content from the tissue compartment using post-processing techniques.[17] FW measured in the WM tissue (WM-FW) has been found to be strongly associated with cognitive status. Specifically, high WM-FW is associated with vascular risk factors (VRFs) and has been linked to a decline in episodic memory (EM) and EF in older individuals.[18,19]

In the present study, we hypothesized that lower ALPS index indicated the dysfunction of glymphatic clearance that would result in the accumulation of FW content in the extracellular space, leading to cognitive decline in EF. Using four cohorts including middle-aged to older individuals with racial and ethnic diversities, we first examined the associations between the ALPS index and EF performance, and then investigated the role of WM-FW as a potential mediator of the relationship between ALPS index and EF performance.

RESEARCH IN CONTEXT

1. **Systematic review**: The glymphatic system (GS) is a discovered putative clearance pathway in the central nervous system. The GS dysfunction was thought to be the probable etiology of cerebral small vessel disease (cSVD) and vascular cognitive impairment and dementia (VCID). However, how changes in the GS affect the cognitive function in cSVD is still unclear.
2. **Interpretation**: In this study, we found that glymphatic clearance dysfunction, as reflected by the ALPS, was strongly associated with impaired executive function (EF), and this association is mediated by interstitial fluid (ISF) drainage in white matter (WM) (increased WM-free water [FW]) in the middle to aged subjects at risk of cSVD.
3. **Future directions**: Our findings provide a clinical rationale for the use of ALPS as a marker of cognitive function in multi-site observational studies and clinical trials to monitor and prevent VCID.

2 | METHODS

2.1 | Participants

Four independent cohorts were included in this study with respective local Institutional Review Boards (IRB) approval.The MarkVCID cohort which consists of seven sites (Johns Hopkins University School of Medicine [JHU], Rush University Medical Center/Illinois Institute of Technology [RUSH], Universities of California San Francisco, Davis, and Los Angeles [UCSF/UCD/UCLA], University of Kentucky [UKY], University of New Mexico Health Science Center [UNM], University of Southern California [USC], and University of Texas Health Science Center at San Antonio [UTHSCSA]). Recruitment sources and inclusion and exclusion criteria for each cohort are summarized in Table S1. A detailed description of the MarkVCID approach for participant enrollment, clinical and cognitive testing, and sample collection can be found elsewhere[20] Three legacy cohorts were from University of California Davis Alzheimer Disease Research Center (UCD_ADRC), University of California San Francisco Memory and Aging Center (UCSF_MAC), and Framingham Heart Study (FHS), respectively. Briefly, the UCD_ADRC cohort includes individuals recruited at the UCD_ADRC, with approximately 74% of the participants recruited through community-based recruitment protocols designed to enhance racial and ethnic diversity and the spectrum of cognitive dysfunction with an emphasis on normal cognition and mild cognitive impairment (MCI).[21] The UCSF_MAC cohort includes community-dwelling older adults with normal cognition or MCI recruited from the UCSF_MAC during the 2-year discovery phase of the MarkVCID consortium.[22] FHS is a three-generation, single-site, community-based, ongoing cohort study initiated in 1948 to investigate the risk factors for cardiovascular disease risk factors. The present study includes individuals from the Offspring and Gen3 cohorts.[23]

The data used in this study excluded the participants with unstable major medical illness, major primary psychiatric disorder, prevalent stroke at MRI assessment, or other neurological disorders that might confound the assessment of cSVD-related VCID.

2.2 | MRI acquisition

The MarkVCID DTI protocol used a single-shell ($b = 1000$ s/mm^2), 40-direction diffusion sequence with a voxel size of $2.0 \times 2.0 \times 2.0$ mm^3 and six $b = 0$ s/mm^2. A separate scan using a reverse-polarity phase encoding gradient was acquired and used to estimate and correct image distortion in the DTI data. The other three cohorts used a single-shell DTI acquisition, with non-b-zero values equal to 1000 s/mm^2. Studies used a voxel size of $2.0 \times 2.0 \times 2.0$ mm^3 for the UCD_ADRC and UCSF_MAC cohorts, and $1.8 \times 1.8 \times 5$ mm^3 for the FHS cohort. The detailed DTI acquisition parameters are summarized in Table S2.

2.3 | ALPS pipeline

The pipeline for ALPS index calculation was described previously.[10] Briefly, the 4D DTI volume DICOM files were converted to NIFTI files using MRIcroGL GUI. Then, an in-house bash script (http://loft-lab.org/index-5.html) was used to compute the ALPS index using DTI images

TABLE 1 Participants' demographics, clinical factors, imaging features, and cognitive status

						Vascular risk factors			Imaging biomarkers for cSVD			Cognitive outcome
Cohort	Total sample size, *n*	Age, years old (mean ± SD)	Sex (F), %	Race/ethnicity, *n* (%)	Education, years (mean ± SD or *n*[%])	Diabetes (Y), %	Hypertension (Y), %	Smoking (Y), %	WM-FW	ALPS index	WMHV	UDS3-EF scores
MarkVCID	547	72.6 ± 7.2	331/547, 61%	Non-Hispanic White: 241/547, 44%; Black/African American: 39/547, 7%; Hispanic ethnicity: 198/547, 36%	15.1 ± 3.5	87/443, 20%	237/450, 53%	171/447, 38%	0.2 ± 0.05	1.2 ± 0.2	0.004 ± 0.004	−0.6 ± 0.9
UCD_ADRC	342	75.5 ± 7.6	229/342, 67%	Non-Hispanic White: 140/342, 41% Black/African American: 91/342, 27% Hispanic ethnicity: 98/342, 29%	14.4 ± 4.1	111/342, 32%	220/342, 64%	151/342, 44%	0.2 ± 0.05	1.0 ± 0.2	0.007 ± 0.010	−0.9 ± 0.9
UCSF_MAC	179	70.2 ± 10.8	96/179, 54%	White (no Hispanic/non-Hispanic distinction): 158/179, 89% Black/African American: 5/179, 2.8%	17.8 ± 2.8	8/118, 7%	45/118, 38%	54/118, 46%	0.2 ± 0.04	1.2 ± 0.1	0.005 ± 0.007	−0.6 ± 1.1
FHS	2682	56.0 ± 13.6	1437/2682 54%	Non-Hispanic White: 1754/2682, 65%; Black/African American: 0/2682, 0%; Hispanic ethnicity: not collected	No high school: 19/2682, 0.7% High school: 420/2682, 15% College: 2243/2682, 84%	193/2682, 7%	884/2682, 33%	200/2682, 7%	0.2 ± 0.03	1.3 ± 0.1	0.002 ± 0.004	−0.2 ± 0.7

Abbreviations: ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0).

TABLE 2 Linear regression and mediation results for the association between ALPS index and EF

	Model 1			Model 2			Model 3		
Parameter	***β***	**95% CI**	***p*-value**	***β***	**95% CI**	***p*-value**	***β***	**95% CI**	***p*-value**
MarkVCID cohort									
ALPS→WM-FW	−0.31	−0.42 to −0.26	<0.001***	−0.30	−0.39 to −0.22	<0.001***	−0.22	−0.32 to −0.17	<0.001***
WM-FW→UDS3-EF	−0.19	−0.29 to −0.08	0.001**	−0.18	−0.29 to −0.07	0.001**	−0.20	−0.32 to −0.07	0.001**
ALPS→UDS3-EF									
Total effect	0.13	0.06 to 0.25	<0.001***	0.11	0.03 to 0.23	0.01*	0.10	0.03 to 0.22	0.02*
Direct effect	0.08	−0.004 to 0.19	0.07	0.07	−0.02 to 0.19	0.17	0.06	−0.02 to 0.17	0.23
Indirect effect	0.06	0.02 to 0.10	<0.001***	0.05	0.02 to 0.09	<0.001***	0.04	0.01 to 0.09	0.002**
UCD_ADRC cohort									
ALPS→WM-FW	−0.12	−0.21 to −0.04	0.005**	−0.12	−0.21 to −0.04	0.004**	−0.11	−0.18 to −0.03	0.004**
WM-FW→UDS3-EF	−0.26	−0.37 to −0.15	<0.001***	−0.26	−0.37 to −0.15	<0.001***	−0.22	−0.35 to −0.08	0.002**
ALPS→UDS3-EF									
Total effect	0.10	0.009 to 0.19	0.02*	0.10	0.008 to 0.19	0.02*	0.09	0.0005 to 0.18	0.05
Direct effect	0.07	−0.02 to 0.16	0.12	0.07	−0.02 to 0.15	0.11	0.06	−0.02 to 0.16	0.16
Indirect effect	0.03	0.008 to 0.06	0.01*	0.03	0.009 to 0.06	0.01*	0.02	0.005 to 0.05	0.004**
UCSF_MAC cohort									
ALPS→WM-FW	−0.26	−0.41 to −0.12	<0.001***	−0.23	−0.40 to −0.07	0.007**	−0.19	−0.35 to −0.04	0.02*
WM-FW→UDS3-EF	−0.31	−0.43 to −0.18	<0.001***	−0.35	−0.52 to −0.18	<0.001***	−0.33	−0.52 to −0.15	<0.001***
ALPS→UDS3-EF									
Total effect	0.23	0.11 to 0.36	<0.001***	0.21	0.040 to 0.36	0.02*	0.19	0.03 to 0.34	0.02*
Direct effect	0.15	0.04 to 0.28	0.012*	0.13	−0.03 to 0.27	0.09	0.13	−0.03 to 0.28	0.12
Indirect effect	0.08	0.03 to 0.14	0.002**	0.08	0.02 to 0.17	0.01*	0.06	0.02 to 0.15	0.03*
FHS cohort									
ALPS→WM-FW	−0.17	−0.19 to −0.14	<0.001***	−0.16	−0.19 to −0.13	<0.001	−0.14	−0.16 to −0.11	0.001**
WM-FW→UDS3-EF	−0.17	−0.22 to −0.12	<0.001***	−0.16	−0.21 to −0.12	<0.001	−0.18	−0.23 to −0.13	<0.001***
ALPS→UDS3-EF									
Total effect	0.07	0.04 to 0.10	<0.001***	0.06	0.03 to 0.10	<0.001***	0.06	0.03 to 0.1	<0.001***
Direct effect	0.04	0.008 to 0.08	0.01*	0.04	0.005 to 0.07	0.036*	0.04	0.001 to 0.08	<0.05*
Indirect effect	0.03	0.02 to 0.04	<0.001***	0.03	0.02 to 0.04	<0.001***	0.02	0.02 to 0.03	<0.001***

Model1: regressing out Demographics, Model2: regressing out Demographics + VRFs, Model3: regressing out Demographics + VRFs + WMHV.
Abbreviations: ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0); WM-FW, mean free water in the white matter; WMHV, white matter hyperintensity volume log-transformed and normalized by total intracranial volume.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

as input and including FMRIB Software Library (FSL) and MRtrix3 commands. The DTI images underwent pre-processing, including denoising and Gibbs-unringing, susceptibility-induced distortion, eddy currents, and movement corrections, then the fractional anisotropy (FA) map, mean diffusivity (MD) map and *x*-, *y*-, and *z*-axis diffusivity maps (Dxx, Dyy, Dzz) were generated after Tensor fitting. The FA map was co-registered to the JHU-ICBM-FA template and the transformation matrix was applied to the MD, Dxx, Dyy, and Dzz maps. The projection and association fibers at the level of the lateral ventricle body were recognized as the superior corona radiata (SCR) and the superior longitudinal fasciculus (SLF) based on the JHU-ICBM-DTI-81-White-Matter Labeled Atlas. The regions of interest (ROIs) were defined as spheres with 5 mm diameter and were placed in the areas of bilateral SCR and SLF which were applied on all MD, Dxx, Dyy, and Dzz maps and further amended to avoid the enlarged lateral ventricle or subcortical regions. The ALPS index was calculated as follows: $\textbf{\textit{ALPS index}} = \frac{Mean\ (Dxxproj,\ Dxxassoc)}{Mean\ (Dyyproj,\ Dzzassoc)}$. The ALPS indices of the left and right hemispheres were calculated separately. We used the

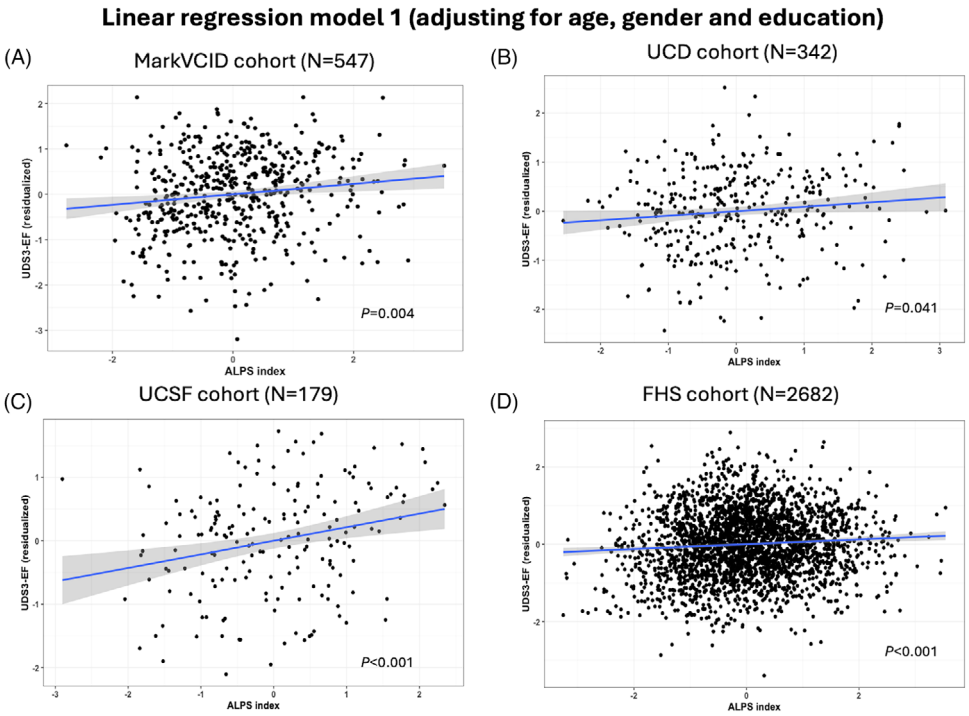

FIGURE 1 Cross-sectional association between the diffusion analysis along PVS (ALPS) index and the validated UDS3-EF in MarkVCID cohort (A), UCD_ADRC cohort (B), UCSF_MAC cohort (C), and FHS cohort (D). Scatter plots show a significantly positive correlation between the ALPS index and residualized UDS3-EF scores for multiple linear regression Model 1, adjusted by demographics, that is, age, gender, and education in all four cohorts (MarkVCID, $p < 0.01$; UCD_ADRC, $p < 0.05$; UCSF_MAC, $p < 0.001$; FHS, $p < 0.001$). ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; PVS, perivascular space; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0); VCID, vascular cognitive impairment and dementia; VRFs, vascular risk factors; WMHV, white matter hyperintensity volume.

average ALPS index of the bilateral hemisphere in the following statistical analysis. The MD and FA were calculated by the mean MD value and the mean FA value in the ROIs of bilateral projection and association fibers, respectively. The radial asymmetry ($\lambda 2/\lambda 3$) patterns of projection fibers ($\lambda 2/\lambda 3$proj) and association fibers ($\lambda 2/\lambda 3$assoc) were calculated by the ratio of the mean Dxx to the mean Dyy in the ROIs of bilateral projection fibers and the ratio of the mean Dxx to the mean Dzz in the ROIs of bilateral association fibers, respectively.

2.4 | FW kit

The preprocessed DTI volumes, brain masks, and files containing the *b*-vector and *b*-values for each volume were input into the MarkVCID FW kit.[24] The model considers two coexisting compartments per voxel: one compartment is an FW compartment, which models isotropic diffusion with a diffusion coefficient of water at body temperature (37°C) fixed to 3×10^{-3} mm^2/s and a second compartment accounts for all other molecules (i.e., intra- and extra-cellular molecules) that are hindered or restricted by physical barriers (e.g., axonal membranes, myeline). The kit generates a measure of mean FW within the WM tissue. WM hyperintensity volume (WMHV) and total intracranial volume (ICV) were also computed using automated procedures as described previously.[25] WMHV was defined as WMH volume log-transformed and normalized by total ICV.

2.5 | Cognitive outcome

The cognitive outcome in this study used a composite measure of EF derived using item-response theory (IRT) generated score, given its consistent associations with cSVD-related cognitive impairment and WM injuries.[26] The composite scores of EF (UDS3-EF) incorporate scores from the following National Alzheimer's Coordinating Center's Uniform Data Set (NACC-UDS) Version 3 neuropsychological tests[26]: Trail Making test, Part B (number of correct lines per minute), Number Span Back-ward (total score), Phonemic fluency (number of correct F-words generated in 1 min), and Category fluency (number of correct responses generated in 1 min).

2.6 | Statistical analysis

All the statistical analyses were performed using RStudio software. Mean ± standard deviation (SD) and frequency (%) are presented for

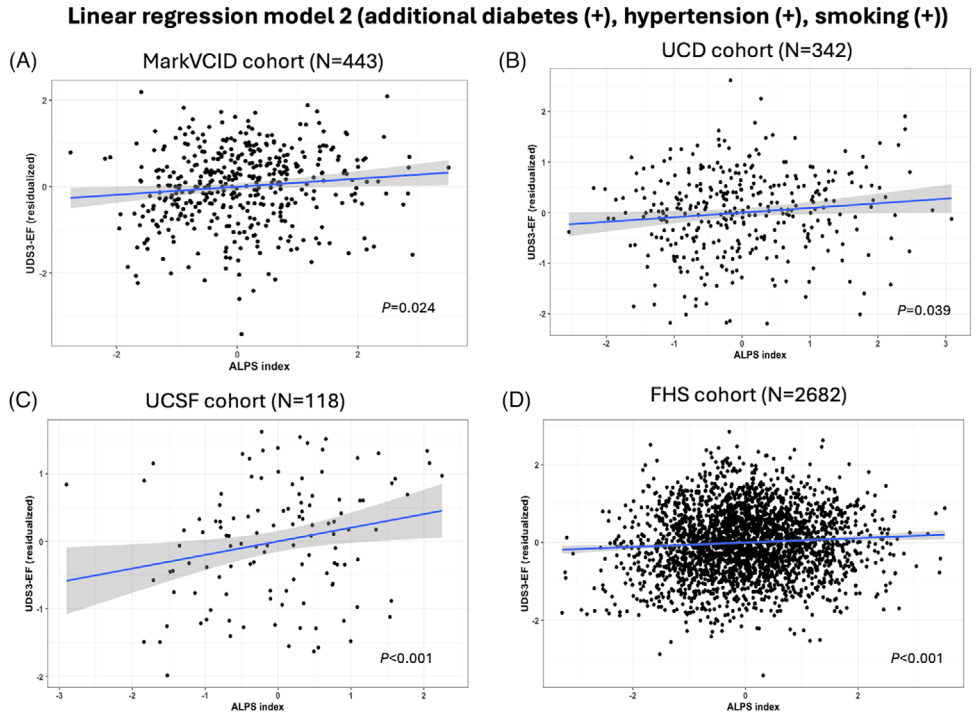

FIGURE 2 Cross-sectional association between the diffusion analysis along PVS (ALPS) index and the validated UDS3-EF in MarkVCID cohort (A), UCD_ADRC cohort (B), UCSF_MAC cohort (C), and FHS cohort (D). Scatter plots show a significantly positive correlation between the ALPS index and residualized UDS3-EF scores for multiple linear regression Model 2, adjusted by additional VRFs that is, the presence of diabetes, hypertension, and smoking to Model 1 in all four cohorts (MarkVCID, $p < 0.05$; UCD_ADRC, $p < 0.05$; UCSF_MAC, $p < 0.001$; FHS, $p < 0.001$). ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; PVS, perivascular space; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0); VCID, vascular cognitive impairment and dementia; VRFs, vascular risk factors; WMHV, white matter hyperintensity volume.

continuous variables and categorical variables, respectively. Multiple linear regression models were used to evaluate the associations of the ALPS index with UDS3-EF scores, regressing out four types of covariates: (1) demographics, that is, age, sex, and education (Model 1). (2) Added VRFs, that is, the presence of diabetes, hypertension, and smoking to Model 1 (Model 2). This second model aimed to estimate the additional contribution of ALPS above VRFs in explaining EF. (3) To further assess the contribution of the WMH burden to UDS3-EF, we added WMHV to the linear regression Model 2 (Model 3) in all four cohorts. (4) To further test the contributions of the DTI-derived metrics indicating WM structural integrity, that is, MD and FA as well as the radial asymmetry ($\lambda 2/\lambda 3$), MD, FA, $\lambda 2/\lambda 3$proj and $\lambda 2/\lambda 3$assoc were first residualized against ALPS index and WM-FW to remove their co-linear effects (ALPS index—MD, $\beta = -0.44$, $p < 0.001$; ALPS index—FA, $\beta = 0.16$, $p < 0.001$; ALPS index—$\lambda 2/\lambda 3$_proj, $\beta = 0.85$, $p < 0.001$; ALPS index—$\lambda 2/\lambda 3$_proj, $\beta = 0.69$, $p < 0.001$; MD—WM-FW, $\beta = 0.75$, $p < 0.001$; FA—WM-FW, $\beta = -0.47$, $p < 0.001$; $\lambda 2/\lambda 3$_proj—WM-FW, $\beta = -0.22$, $p < 0.001$; $\lambda 2/\lambda 3$_assoc—WM-FW, $\beta = -0.55$, $p < 0.001$), which were then included in the linear regression Model 3 and applied on MarkVCID cohort (Model 4). Models' integrities were examined using Cook's distance plot for potential outliers. We further tested the mediation effect of WM-FW on the relationship betweenALPS index and UDS3-EF scores from all regression models using mediation analyses with a mediation package.[27,28] The 95% confidence interval of the mediator effect was estimated with 1000 bootstrapping samples. For bootstrap tests, the p-value of the indirect effect was computed as the smallest significance level α corresponding to the confidence interval obtained from the bootstrapped distribution, which did not contain 0.[28]

3 | RESULTS

3.1 | Demographics

A total of 547 subjects in the MarkVCID consortium, 342 subjects in the UCD_ADRC cohort, 179 subjects in the UCSF_MAC cohort, and 2682 subjects in the FHS cohort were included in this study. Table 1 summarizes the subjects' demographics, clinical characteristics, imaging features, and UDS3-EF scores for the different cohorts used in this study. The average age ranged from 56 to 76 years old. Participants were predominantly female. The cohorts were found to be very diverse in terms of race/ethnicity (UCSF_MAC and FHS cohorts have a higher percentage of non-Hispanic White [NHW] subjects) (65%-89%) and a small proportion of individuals with diabetes (7%), hypertension (33%–38%), and smoking (7%–46%) history. Participants in the FHS cohort

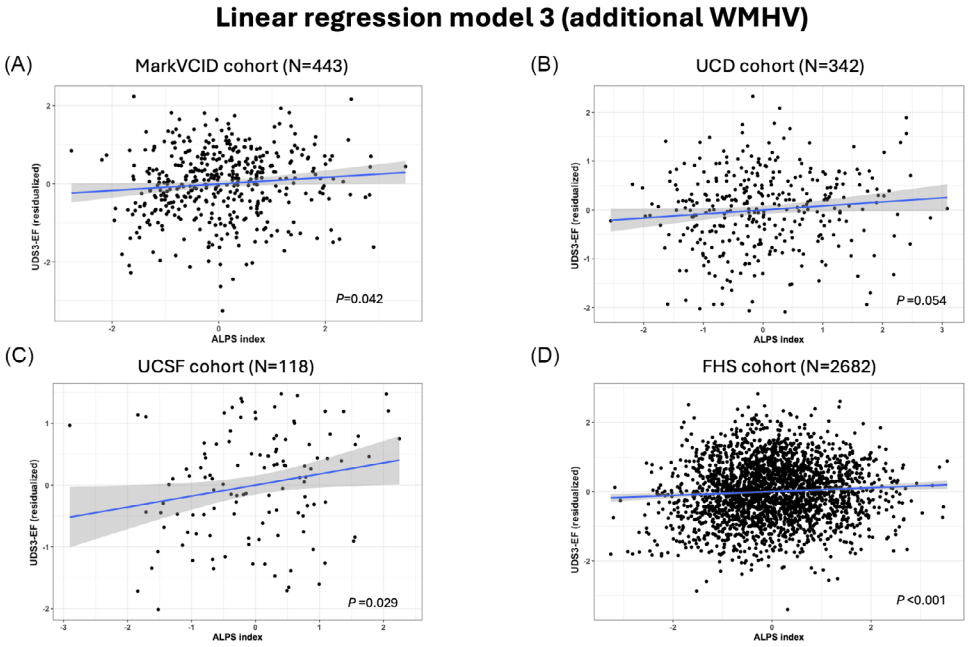

FIGURE 3 Cross-sectional association between the diffusion analysis along PVS (ALPS) index and the validated UDS3-EF in MarkVCID cohort (A), UCD_ADRC cohort (B), UCSF_MAC cohort (C), and FHS cohort (D). Scatter plots show a significantly positive correlation between the ALPS index and residualized UDS3-EF scores for multiple linear regression Model 3, adjusted by additional WMHV to Model 2 in all four cohorts (MarkVCID, $p < 0.05$; UCD_ADRC, $p = 0.054$; UCSF_MAC, $p < 0.001$; FHS, $p < 0.001$). ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; PVS, perivascular space; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0); VCID, vascular cognitive impairment and dementia; VRFs, vascular risk factors; WMHV, white matter hyperintensity volume.

had better EF performance (i.e., higher UDS3-EF scores) compared to those from the other groups.

3.2 | Association between the diffusion along PVS and the EF

The demographics, VRFs and WMH burden were gradually included in the linear regression model as covariates, as they were demonstrated to influence the ALPS index.[29] All participants in the UCD_ADRC and FHS cohorts were included in Model 1, Model 2, and Model 3. All participants in MarkVCID and UCSF_MAC cohorts were included in Model 1, and a subset of 443 subjects of the MarkVCID cohort and 118 subjects of the UCSF_MAC cohort were included in Model 2 and Model 3 due to the missing data on the VRFs. Linear regression analyses revealed that ALPS index was independently associated with UDS3-EF scores in the four cohorts (Table 2) for Model 1 (MarkVCID cohort, $\beta = 0.13$, $p < 0.001$; UCD_ADRC cohort, $\beta = 0.10$, $p < 0.05$; UCSF_MAC cohort, $\beta = 0.23$, $p < 0.001$; FHS cohort, $\beta = 0.07$, $p < 0.001$), Model 2 (MarkVCID cohort, $\beta = 0.11$, $p < 0.05$; UCD_ADRC cohort, $\beta = 0.10$, $p < 0.05$; UCSF_MAC cohort, $\beta = 0.21$, $p < 0.05$; FHS cohort, $\beta = 0.06$, $p < 0.001$), and Model 3 (Mark VCID cohort, $\beta = 0.10$, $p < 0.05$; UCD_ADRC cohort, $\beta = 0.09$, $p = 0.054$; UCSF_MAC cohort, $\beta = 0.19$, $p < 0.05$; FHS cohort, $\beta = 0.06$, $p < 0.001$). Figures 1–3 illustrate the association between the ALPS index and UDS3-EF scores residualized against relevant covariates for all regression models across the four cohorts. A subset of 443 subjects in the MarkVCID cohort were further analyzed using the regression Model 4, ALPS index was demonstrated to be independently associated with UDS3-EF scores, further adjusting for residualized FA value, MD value, $\lambda 2/\lambda 3$proj, and $\lambda 2/\lambda 3$assoc ($\beta = 0.13$, $p < 0.01$) (Table S3). Figure S1 illustrates the association between the ALPS index and UDS3-EF scores residulized against additional markers of WM structural integrity (MD, FA) and WM radial asymmetry ($\lambda 2/\lambda 3$) in the MarkVCID cohort.

3.3 | Mediation effect of mean FW in WM on the relationship between the diffusion along PVS and the executive performance

The mediation analyses included ALPS index as the independent variable, UDS3-EF scores as the dependent variable, and WM-FW as the mediator for all regression models for each cohort. The mediation analyses revealed that WM-FW had a significant mediation effect on the association between ALPS index and UDS3-EF scores in all four cohorts for Model 1 (MarkVCID cohort, indirect effect $\beta = 0.06$, $p < 0.001$, mediation effect 42.6%; UCD_ADRC cohort, indirect effect $\beta = 0.03$, $p < 0.05$, mediation effect 30.5%; UCSF_MAC cohort, indirect effect $\beta = 0.08$, $p < 0.01$, mediation effect 29.4%; FHS cohort, indirect effect $\beta = 0.03$, $p < 0.001$, mediation effect 41.4%), Model 2 (MarkVCID cohort, indirect effect $\beta = 0.05$, $p < 0.001$, mediation effect 42.4%; UCD_ADRC cohort, indirect effect $\beta = 0.03$, $p < 0.05$, mediation effect

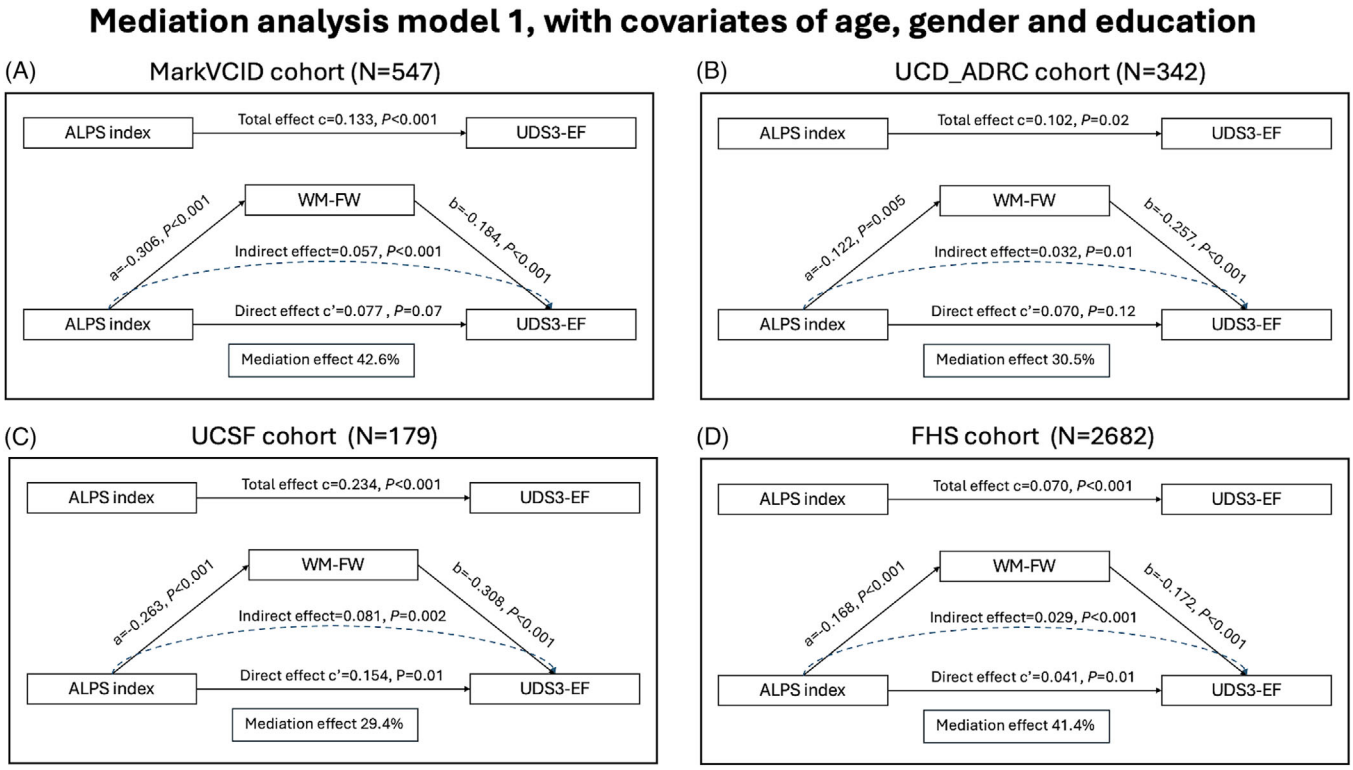

FIGURE 4 Mediation analysis between diffusion analysis along PVS (ALPS) index and the validated UDS3-EF with WM-FW as a mediator in MarkVCID cohort (A), UCD_ADRC cohort (B), UCSF_MAC cohort (C), and FHS cohort (D). The analysis was performed with covariates of demographics, that is, age, sex, and education (Model 1). The mediation analysis diagrams show a significant indirect effect of WM-FW on mediating the relationship between ALPS index and UDS3-EF scores in all four cohorts (MarkVCID cohort, indirect effect $\beta = 0.06$, $p < 0.001$, mediation effect 42.6%; UCD_ADRC cohort, indirect effect $\beta = 0.03$, $p < 0.05$, mediation effect 30.5%; UCSF_MAC cohort, indirect effect $\beta = 0.08$, $p < 0.01$, mediation effect 29.4%; FHS cohort, indirect effect $\beta = 0.03$, $p < 0.05$, mediation effect 41.4%). There's a significant direct effect of the ALPS index on UDS3-EF scores in the UCSF_MAC cohort (direct effect $\beta = 0.15$, $p < 0.05$) and FHS cohort (direct effect $\beta = 0.04$, $p < 0.05$). ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; PVS, perivascular space; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0); VCID, vascular cognitive impairment and dementia; WM-FW, mean free water in the white matter.

29.4%; UCSF_MAC cohort, indirect effect $\beta = 0.08$, $p < 0.05$, mediation effect 29.4%; FHS cohort, indirect effect $\beta = 0.03$, $p < 0.001$, mediation effect 39.5%), and Model 3 (MarkVCID cohort, indirect effect $\beta = 0.04$, $p < 0.01$, mediation effect 41.4%; UCD_ADRC cohort, indirect effect $\beta = 0.02$, $p < 0.01$, mediation effect 26.1%; UCSF_MAC cohort, indirect effect $\beta = 0.07$, $p < 0.05$, mediation effect 32.0%; FHS cohort, indirect effect $\beta = 0.03$, $p < 0.001$, mediation effect 38.6%) (Table 2 and Figure 4–6), as well as for Model 4 in MarkVCID cohort (indirect effect $\beta = 0.05$, $p < 0.01$, mediation effect 40.0%; Table S3 and Figure S2). A direct effect of ALPS index on UDS3-EF scores was evidenced in the UCSF_MAC cohort for Model 1 (direct effect $\beta = 0.15$, $p < 0.05$), as well as the FHS cohort for Model 1 (direct effect $\beta = 0.04$, $p < 0.05$), Model 2 (direct effect $\beta = 0.04$, $p < 0.05$), and Model 3 (direct effect $\beta = 0.04$, $p < 0.05$), but not in the other cohorts (Table 2 and Figures 4–6).

4 | DISCUSSION

Our study provides strong evidence that the ALPS index is independently associated with UDS3-EF scores, after adjusting for demographics, VRFs, and classical cSVD markers, and that WM-FW mediates this association in four independent middle-to-aged cohorts with racial and ethnic diversities. In addition, because WM microstructural integrity (MD, FA) and WM radial asymmetry pattern ($\lambda 2/\lambda 3$) have been reported to bias the ALPS index and be associated with cognitive impairment,[30–32] we added these two confounders to the regression models and found the association between ALPS index and UDS3-EF scores and the mediation effects of WM-FW remained significant in the MarkVCID cohort, suggesting that microstructural integrity and radial asymmetry pattern of WM fibers did not have much impact on the results. All the above findings extend our previous results showing the

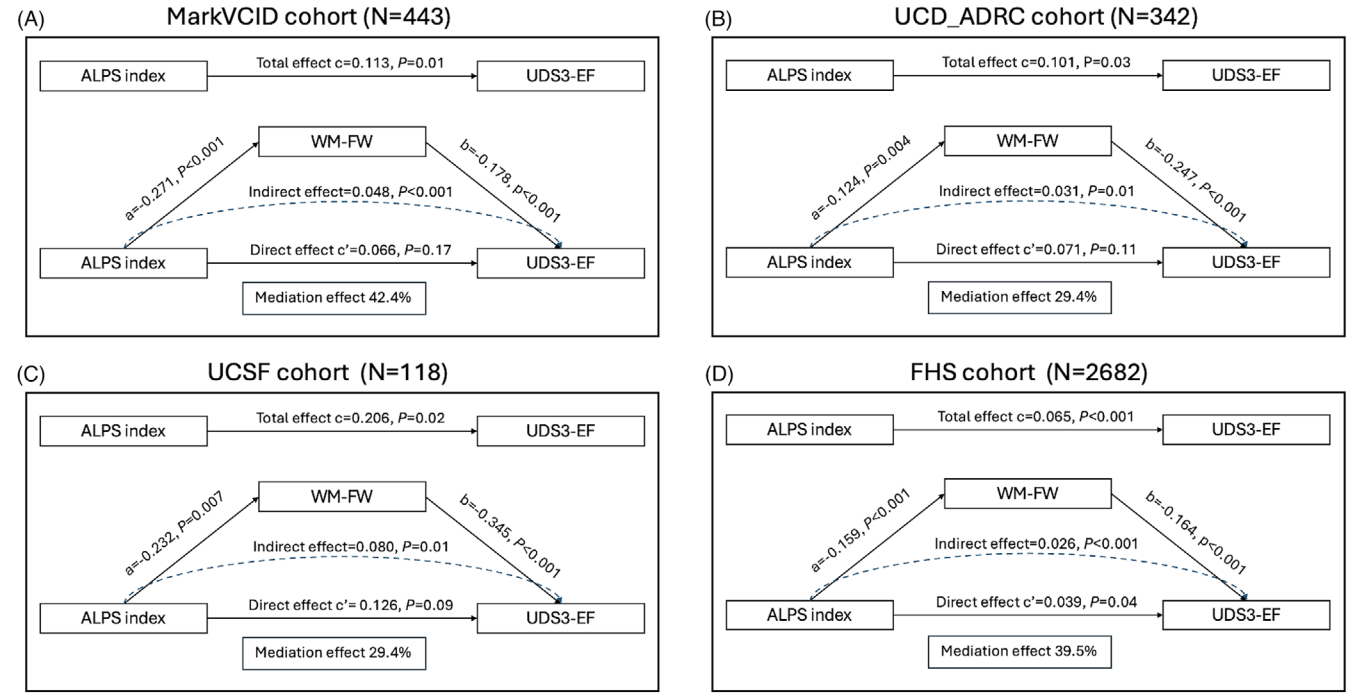

FIGURE 5 Mediation analysis between diffusion analysis along PVS (ALPS) index and the validated UDS3-EF with WM-FW as a mediator in MarkVCID cohort (A), UCD_ADRC cohort (B), UCSF_MAC cohort (C), and FHS cohort (D). The analysis was performed with covariates of additional VRFs that is, the presence of diabetes, hypertension, and smoking to Model 1 (Model 2). The mediation analysis diagrams show a significant indirect effect of WM-FW on mediating the relationship between ALPS index and UDS3-EF scores in all four cohorts (MarkVCID cohort, indirect effect $\beta = 0.05$, $p < 0.001$, mediation effect 42.4%; UCD_ADRC cohort, indirect effect $\beta = 0.03$, $p < 0.05$, mediation effect 29.4%; UCSF_MAC cohort, indirect effect $\beta = 0.08$, $p < 0.05$, mediation effect 29.4%; FHS cohort, indirect effect $\beta = 0.03$, $p < 0.001$, mediation effect 39.5%). There's a significant direct effect of the ALPS index on UDS3-EF scores in the FHS cohort (direct effect $\beta = 0.04$, $p < 0.05$). ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; PVS, perivascular space; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0); VCID, vascular cognitive impairment and dementia; VRFs, vascular risk factors; WM-FW, mean free water in the white matter.

instrumental validity of ALPS, including excellent cross-vendor reproductivity and test–retest repeatability in the MarkVCID cohort,[10] by demonstrating the clinical validity for the use of the ALPS index as a biomarker of cognitive function in cSVD-related VCID.

Animal and human studies have demonstrated that glymphatic dysfunction is involved in the pathophysiology and etiology of cSVD and VCID.[2,7,8,33,34]GS has been proposed as a fluid clearance pathway in CNS. When GS is impaired, waste substances, such as amyloid-beta (Aβ) and tau will be accumulated, leading to neuronal damage and cognitive impairment.[35] In addition, GS carries immune cells and inflammatory factors. Dysfunction of GS might result in neuroinflammation and neuronal damage.[36,37] Furthermore, cSVD contributed to arterial pulsation alterations and AQP4 water channel dislocation, which influences CSF influx of GS that could exacerbate Aβ deposition and cognitive decline.[8,33,38] DTI-ALPS approach is based on the assumption that the glymphatic efflux pathway (perivenous space) runs in the same direction as the medullary veins at the lateral ventricle body level.[9] ALPS index measures the water diffusion capacity along the PVS surrounding the medullary veins, which could reflect part of the glymphatic clearance function (ISF drainage from the WM area) to some extent. A previous study compared the DTI-ALPS method with the gold standard of glymphatic MRI with intrathecal administration of gadolinium and provided the evidence that ALPS index has a good consistency with the glymphatic clearance function.[13] Several studies have demonstrated that a lower ALPS index was related to radiological features and cognitive decline in cSVD patients.[7,13,15,16,39] In this study, we focused on EF, which manifests as the impaired capacity to use complex information, formulate strategies, and exercise self-control. EF is among the first cognitive domains to decline with aging[40] and is a prominent cognitive phenotype of cSVD in which less pronounced EM deficits are present compared to AD patients.[41] Our results showed that the reduced ALPS index was independently associated with the poorer EF in four cohorts of adult and older individuals, suggesting a close relationship between the impairment of ISF drainage and cognitive decline in individuals at risk for cSVD, which is in line with previous studies.

Previous human studies have evidenced that larger DTI-derived metrics of WM integrity were independently associated with lower

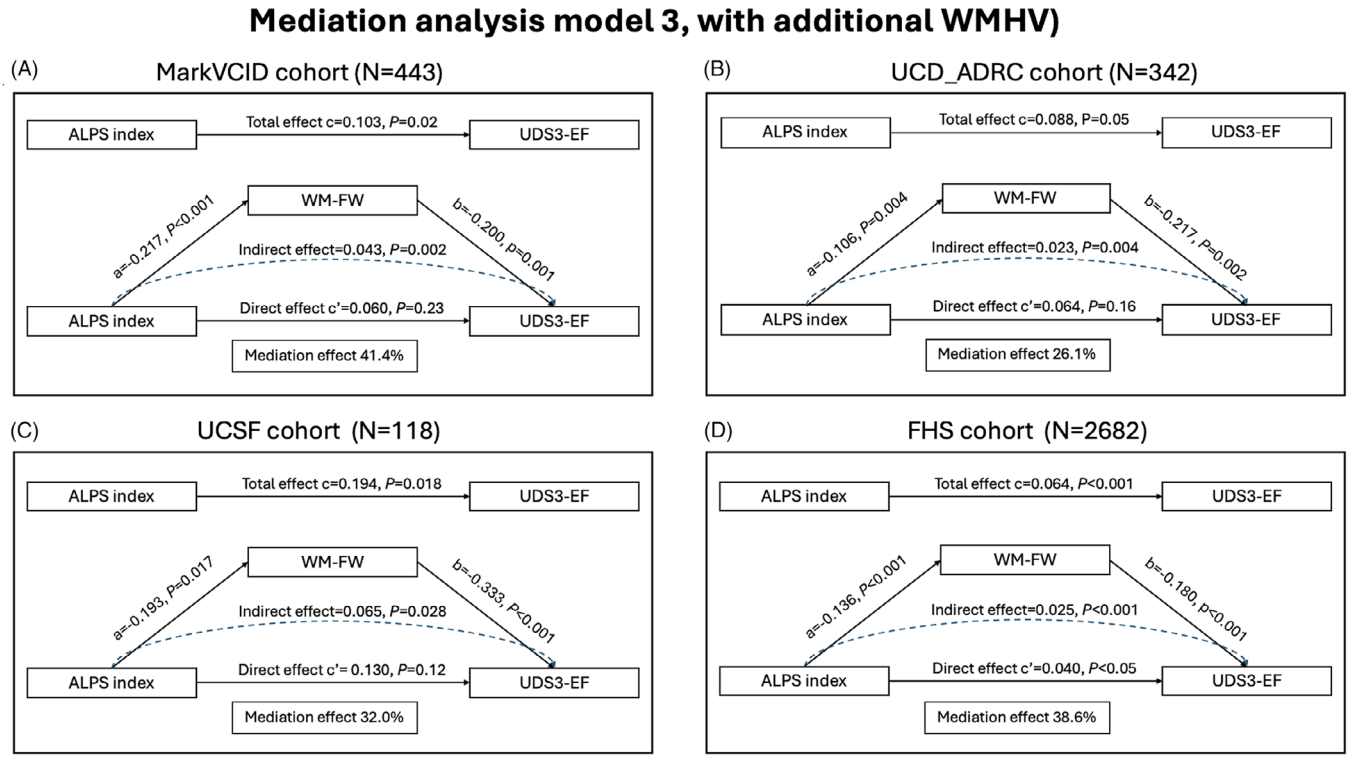

FIGURE 6 Mediation analysis between diffusion analysis along PVS (ALPS) index and the validatedUDS3-EF with WM-FW as mediator in MarkVCID cohort (A), UCD_ADRC cohort (B), UCSF_MAC cohort (C), and FHS cohort (D). The analysis was performed with covariates of additional WMHV to Model 2 (Model 3). The mediation analysis diagrams show a significant indirect effect of WM-FW on mediating the relationship between ALPS index and UDS3-EF scores in all four cohorts (MarkVCID cohort, indirect effect $\beta = 0.05$, $p < 0.01$, mediation effect 41.4%; UCD_ADRC cohort, indirect effect $\beta = 0.02$, $p < 0.001$, mediation effect 26.1%; UCSF_MAC cohort, indirect effect $\beta = 0.07$, $p < 0.05$, mediation effect 32.0%; FHS cohort, indirect effect $\beta = 0.03$, $p < 0.001$, mediation effect 38.6%). There's a significant direct effect of the ALPS index on UDS3-EF scores in the FHS cohort (direct effect $\beta = 0.04$, $p < 0.05$). ALPS, diffusion analysis along the perivascular space; FHS, Framingham Heart Study; UCD_ADRC, University of California Davis Alzheimer's Disease Research Center; UCSF_MAC, University of California San Francisco Memory and Aging Center; UDS3-EF, composite scores of executive functions derived from the Uniform Data Set (v3.0); VCID, vascular cognitive impairment and dementia; WM-FW, mean free water in the white matter; WMHV, white matter hyperintensity volume.

EF in cSVD patients.[42–44] However, DTI-derived metrics were currently thought to be likely contaminated by extracellular water.[17] The FW model, which consists of a FW compartment and a tissue compartment, was proposed to study diffusion changes in WM more accurately.[17,45] The FW compartment represents water molecules that are not restricted or oriented, which can be used as an imaging marker for an ISF fraction.[19] Of importance, numerous recent studies demonstrated that higher WM-FW was strongly associated with cognitive decline in older adults as well as individuals with MCI and AD.[19,46–48] A recent study reported a significant negative association between WM-FW and EF with a processing speed component, specifically in the fornix and the corpus callosum in a cohort of community-dwelling subjects.[46] This study underlined the dominant role of WM-FW as a determinant of EF with a processing speed component, as compared to the WM fiber microstructure integrity.[46] Our previous work has reported a negative relationship between WM-FW and UDS3-EF scores in six independent cohorts, including MarkVCID, UCD_ADRC, UCSF_MAC, and FHS cohorts.[18] In the present study, we further detected that WM-FW significantly mediated the relationship between ALPS index and UDS3-EF scores in the same cohorts. Our observations on the association between the ALPS index and WM-FW suggest a plausible underlying biological mechanism that might involve GS. Lower ALPS index, which indicates the impediment of ISF drainage along the PVS, thus leading to an increase in ISF fraction and WM-FW. It is worth noting that, we utilized single-shell acquisition of DTI data for FW reconstruction in the present study, which requires model simplifications and regularization and, therefore, is sensitive to the initialization parameters and may not distinguish between FW and MD alterations.[49,50] The Multishell approach requires nonlinear minimization techniques with a high computational burden, but can lead to a more robust fit, which can be considered to enhance the accuracy of FW calculation in our future studies.

In this study, we noticed that there's a direct effect of ALPS index on UDS3-EF scores in UCSF_MAC and FHS cohorts in addition to the mediation effect of WM-FW. This variation of mediation effect between UCSF_MAC and FHS cohorts and other cohorts might be due to (1) Difference in racial/ethnicity: compared to other cohorts, UCSF_MAC and FHS cohorts have a higher percentage of NHW subjects and FHS cohorts showed better EF performance. Previous studies have reported the influence of racial and ethnic disparity on cognition

in older adults and AD patients and demonstrated NHW subjects presented less cognitive decline than Hispanic and non-Hispanic Black (NHB) subjects.[51,52] (2) Difference in DTI acquisition parameters and scanner: the voxel size of DTI images in the FHS cohort was different from that in other cohorts (non-isotropic) and part of the DTI data was acquired by the 1.5T MR scanner, which might affect the ALPS index calculation. (3) Difference in baseline cSVD burden: although WMH burden has been considered in this study, other cSVD markers, for example, lacunes, and cerebral microbleeds (CMBs), were not collected and included in the analysis. Further studies should take it into account.

To our knowledge, this is the first study to investigate the mediation role of WM-FW on the relationship between ALPS index and EF in a large sample of middle to aged subjects at risk for cSVD. Although ALPS index was regarded as a surrogate marker of glymphatic clearance function in a couple of previous clinical studies,[53–56] DTI-ALPS method itself has some limitations when interpreting the relationship between ALPS index and the glymphatic function, including (1) ALPS index indicates the capacity of water diffusivity "along" the direction of PVS, not "within" the PVS.[57] (2) Placed ROIs for calculating the ALPS index, which may also be affected by partial volume effects of other sources of directional water motion, for example, other fiber tracts, or confounding factors, for example, patient's motion, blood flow.[57,58] (3) DTI-ALPS method only evaluates the area outside the lateral ventricle body and cannot evaluate perivascular diffusivity in other regions where the PVS does not run in the *x*-, *y*-, *z*-axis directions.[57] While GS has been confirmed to involve various brain regions,[33] to what extent the ALPS index indicates the whole brain glymphatic function is a subject of active debate.[59] In this study, we further investigated the PVS volume fraction in normal-appearing WM and basal ganglia (nawmPVSVF and bgPVSVF, respectively), which is one of the key surrogate markers of ISF drainage and glymphatic function, but we did not detect similar mediation effect of PVSVF on the associations between ALPS index and EF scores to that of WM-FW (see Supplemental Materials). There are other emerging biomarkers of glymphatic function, for example, choroid plexus volume and DTI low-b matrixes, worth testing in our future study. Recently, increasing numbers of studies investigated the associations of ALPS index or WM-FW with cSVD biomarkers, or evaluated the mediating effect of ALPS index or WM-FW on the associations between the amyloid pathology, cSVD biomarkers, cSVD-related pathophysiological changes (e.g., BBB water exchange) and cognitive decline in elderly subjects and cSVD patients.[39,60–62] Although not the focus of the present work, exploring the relationships between the cSVD-related risk factors, amyloid pathology, cSVD pathophysiological changes, and cognitive decline helps to better understand the different pathophysiological biomechanisms and relevant confounding factors involved in cSVD in the context of VCID. Additionally, we did not observe significant findings of longitudinal analysis with a relatively small sample size (a subset of 286 subjects in the MarkVCID cohort) in the study (see Supplemental Materials). Future studies need to increase the sample size to explore the association between ALPS index and the longitudinal EF decline as well as the potential factors that mediate such association.

In conclusion, our findings suggest that, in middle-aged to aged individuals, a lower ALPS index, which may be a surrogate marker of glymphatic dysfunction, is strongly associated with EF and that this association is mediated by the ISF drainage in WM. The findings provide a strong clinical rationale for the use of ALPS index as a marker of cognitive function in multi-site observational studies and clinical trials to monitor and prevent VCID.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interest to disclose. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

The study protocols were approved by Institutional Review Boards (IRB) of the local sites in all four cohorts to ensure ethicality and compliance with regulations. All participants provided signed informed consent prior to study enrollment.

REFERENCES

1. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:2672-2713.
2. Inoue Y, Shue F, Bu G, Kanekiyo T. Pathophysiology and probable etiology of cerebral small vessel disease in vascular dementia and Alzheimer's disease. *Mol Neurodegener*. 2023;18:46.
3. Yang T, Sun Y, Lu Z, Leak RK, Zhang F. The impact of cerebrovascular aging on vascular cognitive impairment and dementia. *Ageing Res Rev*. 2017;34:15-29.
4. Iadecola C, Duering M, Hachinski V, et al. Vascular cognitive impairment and dementia: JACC scientific expert panel. *J Am Coll Cardiol*. 2019;73:3326-3344.
5. Benveniste H, Liu X, Koundal S, Sanggaard S, Lee H, Wardlaw J. The glymphatic system and waste clearance with brain aging: a review. *Gerontology*. 2019;65:106-119.
6. Rasmussen MK, Mestre H, Nedergaard M. The glymphatic pathway in neurological disorders. *Lancet Neurol*. 2018;17:1016-1024.
7. Tang J, Zhang M, Liu N, et al. The association between glymphatic system dysfunction and cognitive impairment in cerebral small vessel disease. *Front Aging Neurosci*. 2022;14:916633.

8. Li M, Kitamura A, Beverley J, et al. Impaired glymphatic function and pulsation alterations in a mouse model of vascular cognitive impairment. *Front Aging Neurosci*. 2022;13:788519.
9. Taoka T, Masutani Y, Kawai H, et al. Evaluation of glymphatic system activity with the diffusion MR technique: Diffusion Tensor Image Analysis Along the Perivascular Space (DTI-ALPS) in Alzheimer's disease cases. *Jpn J Radiol*. 2017;35:172-178.
10. Liu X, Barisano G, Shao X, et al. Cross-vendor test-retest validation of Diffusion Tensor Image Analysis-Along the Perivascular Space (DTI-ALPS) for evaluating glymphatic system function. *Aging Dis*. 2024;15:1885-1898.
11. Taoka T, Ito R, Nakamichi R, et al. Reproducibility of Diffusion Tensor Image Analysis Along the Perivascular Space (DTI-ALPS) for evaluating interstitial fluid diffusivity and glymphatic function: CHanges in Alps index on Multiple conditiON acquIsition eXperiment (CHAMONIX) study. *Jpn J Radiol*. 2022;40:147-158.
12. Saito Y, Kamagata K, Andica C, et al. Reproducibility of automated calculation technique for diffusion tensor image analysis along the perivascular space. *Jpn J Radiol*. 2023;41:947-954.
13. Zhang W, Zhou Y, Wang J, et al. Glymphatic clearance function in patients with cerebral small vessel disease. *Neuroimage*. 2021;238:118257.
14. Tian Y, Cai X, Zhou Y, et al. Impaired glymphatic system as evidenced by low diffusivity along perivascular spaces is associated with cerebral small vessel disease: a population-based study. *Stroke Vasc Neurol*. 2023;8:413-423.
15. Xu J, Su Y, Fu J, et al. Glymphatic dysfunction correlates with severity of small vessel disease and cognitive impairment in cerebral amyloid angiopathy. *Eur J Neurol*. 2022;29:2895-2904.
16. Ke Z, Mo Y, Li J, et al. Glymphatic dysfunction mediates the influence of white matter hyperintensities on episodic memory in cerebral small vessel disease. *Brain Sci*. 2022;12:1611.
17. Pasternak O, Sochen N, Gur Y, Intrator N, Assaf Y. Free water elimination and mapping from diffusion MRI. *Magn Reson Med*. 2009;62:717-730.
18. Maillard P, Hillmer LJ, Lu H, et al. MRI free water as a biomarker for cognitive performance: validation in the MarkVCID consortium. *Alzheimers Dement*. 2022;14:e12362.
19. Maillard P, Fletcher E, Singh B, et al. Cerebral white matter free water: a sensitive biomarker of cognition and function. *Neurology*. 2019;92:e2221-e2231.
20. Wilcock D, Jicha G, Blacker D, et al. MarkVCID cerebral small vessel consortium: I. Enrollment, clinical, fluid protocols. *Alzheimers Dement*. 2021;17:704-715.
21. Carmichael O, Mungas D, Beckett L, et al. MRI predictors of cognitive change in a diverse and carefully characterized elderly population. *Neurobiol Aging*. 2012;33:83-95.
22. Altendahl M, Maillard P, Harvey D, et al. An IL-18-centered inflammatory network as a biomarker for cerebral white matter injury. *PLoS One*. 2020;15:e0227835.
23. Splansky GL, Corey D, Yang Q, et al. The third generation cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *Am J Epidemiol*. 2007;165:1328-1335.
24. Maillard P, Lu H, Arfanakis K, et al. Instrumental validation of free water, peak-width of skeletonized mean diffusivity, and white matter hyperintensities: markVCID neuroimaging kits. *Alzheimers Dement*. 2022;14:e12261.
25. DeCarli C, Fletcher E, Ramey V, Harvey D, Jagust WJ. Anatomical mapping of White Matter Hyperintensities (WMH): exploring the relationships between periventricular WMH, deep WMH, and total WMH burden. *Stroke*. 2005;36:50-55.
26. Staffaroni AM, Asken BM, Casaletto KB, et al. Development and validation of the Uniform Data Set (v3.0) Executive Function composite score (UDS3-EF). *Alzheimers Dement*. 2021;17:574-583.
27. Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. *Psychol Methods*. 2010;15:309-334.
28. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. *J Stat Softw*. 2014;59:1-38.
29. Zhang Y, Zhang R, Ye Y, et al. The influence of demographics and vascular risk factors on glymphatic function measured by diffusion along perivascular space. *Front Aging Neurosci*. 2021;13:693787.
30. Taoka T, Ito R, Nakamichi R, Nakane T, Kawai H, Naganawa S. Diffusion Tensor Image Analysis Along the Perivascular Space (DTI-ALPS): revisiting the meaning and significance of the method. *Magn Reson Med Sci*. 2024;23:268-290.
31. Kamagata K, Andica C, Takabayashi K, et al. Association of MRI indices of glymphatic system with amyloid deposition and cognition in mild cognitive impairment and Alzheimer disease. *Neurology*. 2022;99:E2648-E2660.
32. Wright AM, Wu YC, Chen NK, Wen Q. Exploring radial asymmetry in MR diffusion tensor imaging and its impact on the interpretation of glymphatic mechanisms. *J Magn Reson Imaging*. 2023;60(4):1432-1441.
33. Chen X, Liu X, Koundal S, et al. Cerebral amyloid angiopathy is associated with glymphatic transport reduction and time-delayed solute drainage along the neck arteries. *Nat Aging*. 2022;2:214-223.
34. Kounda S, Elkin R, Nadeem S, et al. Optimal mass transport with lagrangian workflow reveals advective and diffusion driven solute transport in the glymphatic system. *Sci Rep*. 2020;10:1990.
35. Wardlaw JM, Benveniste H, Nedergaard M, et al. Perivascular spaces in the brain: anatomy, physiology and pathology. *Nat Rev Neurol*. 2020;16:137-153.
36. Carotenuto A, Cacciaguerra L, Pagani E, Preziosa P, Filippi M, Rocca MA. Glymphatic system impairment in multiple sclerosis: relation with brain damage and disability. *Brain*. 2022;145:2785-2795.
37. Mentis AFA, Dardiotis E, Chrousos GP. Apolipoprotein E4 and meningeal lymphatics in Alzheimer disease: a conceptual framework. *Mol Psychiatry*. 2021;26:1075-1097.
38. Zeppenfeld DM, Simon M, Haswell JD, et al. Association of perivascular localization of aquaporin-4 with cognition and Alzheimer disease in aging brains. *JAMA Neurol*. 2017;74:91-99.
39. Hong H, Hong L, Luo X, et al. The relationship between amyloid pathology, cerebral small vessel disease, glymphatic dysfunction, and cognition: a study based on Alzheimer's disease continuum participants. *Alzheimers Res Ther*. 2024;16:43.
40. Lacreuse A, Raz N, Schmidtke D, Hopkins WD, Herndon JG. Age-related decline in executive function as a hallmark of cognitive aging in primates: an overview of cognitive and neurobiological studies: aging of executive function in primates. *Philos Trans R Soc Lond B Biol Sci*. 2020;375:20190618.
41. Wallin A, Román GC, Esiri M, et al. Update on vascular cognitive impairment associated with subcortical small-vessel disease. *J Alzheimers Dis*. 2018;62:1417-1441.
42. Tuladhar AM, Van Norden AGW, De Laat KF, et al. White matter integrity in small vessel disease is related to cognition. *Neuroimage Clin*. 2015;7:518-524.
43. Prins ND, Van Dijk EJ, Den Heijer T, et al. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain*. 2005;128:2034-2041.
44. Smith EE, Salat DH, Jeng J, et al. Correlations between MRI white matter lesion location and executive function and episodic memory. *Neurology*. 2011;76:1492-1499.
45. Zhang R, Huang P, Jiaerken Y, et al. Venous disruption affects white matter integrity through increased interstitial fluid in cerebral small vessel disease. *J Cereb Blood Flow Metab*. 2021;41:157-165.
46. Berger M, Pirpamer L, Hofer E, et al. Free water diffusion MRI and executive function with a speed component in healthy aging. *Neuroimage*. 2022;257:119303.

47. Huang P, Zhang R, Jiaerken Y, et al. White matter free water is a composite marker of cerebral small vessel degeneration. *Transl Stroke Res*. 2021;13:56-64.
48. Zhu Z, Zeng Q, Zhang R, et al. White matter free water outperforms cerebral small vessel disease total score in predicting cognitive decline in persons with mild cognitive impairment. *J Alzheimers Dis*. 2022;86:741-751.
49. Nemmi F, Levardon M, Péran P. Brain-age estimation accuracy is significantly increased using multishell free-water reconstruction. *Hum Brain Mapp*. 2022;43:2365-2376.
50. Hoy AR, Koay CG, Kecskemeti SR, Alexander AL. Optimization of a free water elimination two-compartment model for diffusion tensor imaging. *Neuroimage*. 2014;103:323-333.
51. Gupta S. Racial and ethnic disparities in subjective cognitive decline: a closer look, United States, 2015-2018. *BMC Public Health*. 2021;21:1173.
52. Díaz-Venegas C, Downer B, Langa KM, Wong R. Racial and ethnic differences in cognitive function among older adults in the USA. *Int J Geriatr Psychiatry*. 2016;31:1004-1012.
53. Jin Y, Zhang W, Yu M, et al. Glymphatic system dysfunction in middle-aged and elderly chronic insomnia patients with cognitive impairment evidenced by Diffusion Tensor Imaging Along the Perivascular Space (DTI-ALPS). *Sleep Med*. 2024;115:145-151.
54. Hsu JL, Wei YC, Toh CH, et al. Magnetic resonance images implicate that glymphatic alterations mediate cognitive dysfunction in Alzheimer disease. *Ann Neurol*. 2023;93:164-174.
55. Hsiao WC, Chang HI, Hsu SW, et al. Association of cognition and brain reserve in aging and glymphatic function using Diffusion Tensor Image-Along the Perivascular Space (DTI-ALPS). *Neuroscience*. 2023;524:11-20.
56. Chang H-I, Huang C-W, Hsu S-W, et al. Gray matter reserve determines glymphatic system function in young-onset Alzheimer's disease: evidenced by DTI-ALPS and compared with age-matched controls. *Psychiatry Clin Neurosci*. 2023;77:401-409.
57. Taoka T, Ito R, Nakamichi R, Nakane T, Kawai H, Naganawa S. Diffusion Tensor Image Analysis Along the Perivascular Space (DTI-ALPS): revisiting the meaning and significance of the method. *Magn Reson Med Sci*. 2024;3:268-290.
58. Ringstad G. Glymphatic imaging: a critical look at the DTI-ALPS index. *Neuroradiology*. 2024;66:157-160.
59. Agarwal N, Lewis LD, Hirschler L, et al. Current understanding of the anatomy, physiology, and magnetic resonance imaging of neurofluids: update from the 2022 "ISMRM imaging neurofluids study group" workshop in Rome. *J Magn Reson Imaging*. 2024;59:431-449.
60. Li H, Jacob MA, Cai M, et al. Perivascular spaces, diffusivity along perivascular spaces, and free water in cerebral small vessel disease. *Neurology*. 2024;102:e209306.
61. Hsu SL, Liao YC, Wu CH, et al. Impaired cerebral interstitial fluid dynamics in cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy. *Brain Commun*. 2024;6:fcad349.
62. Ji F, Chai YL, Liu S, et al. Associations of blood cardiovascular biomarkers with brain free water and its relationship to cognitive decline: a diffusion-mri study. *Neurology*. 2023;101:e151-e163.

SUPPORTING INFORMATION

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

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