

FEATURED ARTICLE

Probing the proteome to explore potential correlates of increased Alzheimer's-related cerebrovascular disease in adults with Down syndrome

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Abstract

Cerebrovascular disease is associated with symptoms and pathogenesis of Alzheimer's disease (AD) among adults with Down syndrome (DS). The cause of increased dementia-related cerebrovascular disease in DS is unknown. We explored whether protein markers of neuroinflammation are associated with markers of cerebrovascular disease among adults with DS. Participants from the Alzheimer's disease in Down syndrome (ADDs) study with magnetic resonance imaging (MRI) scans and blood biomarker data were included. Support vector machine (SVM) analyses examined the relationship of blood-based proteomic biomarkers with MRI-defined cerebrovascular disease among participants characterized as having cognitive decline ($n = 36$, mean age \pm SD = 53 ± 6.2) and as being cognitively stable ($n = 78$, mean age = 49 ± 6.4). Inflammatory and AD markers were associated with cerebrovascular disease, particularly among symptomatic individuals. The pattern suggested relatively greater

inflammatory involvement among cognitively stable individuals and greater AD involvement among those with cognitively decline. The findings help to generate hypotheses that both inflammatory and AD markers are implicated in cerebrovascular disease among those with DS and point to potential mechanistic pathways for further examination.

1 | NARRATIVE

1.1 | Contextual background

Adults with Down syndrome (DS) are at increased risk for developing Alzheimer's disease (AD). By their 50s, most have amyloid beta ($A\beta$) and tau-related pathology and almost all have symptoms of dementia by age 70.¹ The study of the pathophysiology of AD among adults with DS is important for two reasons. First, with improved medical management, individuals with DS are living longer than in the past.^{2,3} Because AD incidence is age dependent, the clinical impact of increased longevity amplifies a growing public health crisis for this population. Second, as with autosomal dominant forms of AD,⁴ in DS, AD shares pathophysiological features with late-onset AD, and therefore, examination of AD in adults with DS has the potential to inform our understanding of the disease in the neurotypical population.

There is much debate about the role of cerebrovascular disease in AD. Cerebrovascular disease is also age dependent, common, and co-occurs with AD pathology more often than not among persons diagnosed clinically with AD.⁵ One view is that cerebrovascular disease is a prevalent comorbidity that contributes additively to the clinical presentation of AD. Under this conceptualization, exposure to common vascular risk factors, like hypertension and diabetes, can promote small vessel ischemic cerebrovascular lesions, which, in turn, contribute to clinical symptoms. Another is that cerebrovascular disease is fundamental to AD pathogenesis and therefore may interact on a system level with other core pathologies to exacerbate disease progression or onset. Here, cerebrovascular dysfunction is not solely related to vascular risk factors, although exposure to these factors can exacerbate or amplify their severity and subsequent impact on AD course.

Previous work established that magnetic resonance imaging (MRI) vascular brain injury markers, like white matter hyperintensities (WMH), cerebral microbleeds, and lacunar infarcts are associated with AD risk and progression in the neurotypical population.⁶ Our own work showed that WMH volume is elevated in individuals with autosomal dominant gene mutations for AD up to 20 years prior to the expected symptom onset.⁷ This effect was not statistically mediated by cerebral amyloid angiopathy,⁸ and was independent of systemic vascular risk factors, suggesting a primary role of cerebrovascular disease in AD that is independent of vascular amyloid pathology. Our recent study within the Alzheimer's disease in Down syndrome (ADDS) project⁹ found that cerebrovascular lesions—including WMH, enlarged perivascular spaces (PVS), infarcts, and microbleeds (which are present to a greater extent in DS¹⁰)—were detectable among adults with DS as early as age

40. These markers generally increased monotonically across diagnostic categories, with cognitively stable adults with DS evidencing the lowest severity, followed by those with mild cognitive impairment (MCI), those with possible AD dementia, and those with definite AD dementia. Compared with the neurotypical population, traditional vascular risk factors, like hypertension and atherosclerosis, are rare in individuals with DS.^{11–14} In our previous study, only 7% and 6% of participants, respectively, had hypertension and type 2 diabetes.¹⁵

If the association between cerebrovascular lesions and AD is not attributable solely to exposure to vascular risk factors, then what factors are mediating this effect? There is recent recognition of a potential role of inflammatory drivers in AD pathogenesis^{16,17} and of complex interactions with small vessel and immunological integrity.^{18,19} Both proinflammatory and anti-inflammatory pathways are implicated in AD,²⁰ and there is emerging evidence of a unique neuroinflammatory profile related to AD in adults with DS.^{21–23} We previously found that proteomic profiles discriminate among those with preclinical AD, prodromal AD, and dementia.²⁴

The purpose of this study was to explore the relationship of peripheral, blood-based proteomic neuroinflammatory, neuroimmunological, vascular, and AD-related biomarkers with MRI markers of vascular brain injury that differ across diagnostic groups among adults with DS.⁹ Although we had the overarching hypothesis that more extensive cerebrovascular pathology would be associated with markers of inflammation, we did not generate *a priori* hypotheses about which specific proteomic markers would be associated. Rather, we took an exploratory approach to test the possibility that inflammatory markers and AD-related biomarkers are implicated in cerebrovascular disease in adults with DS who are cognitively stable or exhibiting cognitive decline. This work follows up on our previous observations in ADDS⁹ to examine the possibility that plasma proteomic markers of inflammation and AD are related to neuroimaging markers of cerebrovascular disease in adults with DS who are cognitively stable or exhibit cognitive decline. Given that adults with DS have low prevalence of vascular risk factors,^{11,12} our approach allowed us to gain insights into potential pathways toward developing cerebrovascular disease that are independent of traditional vascular risk factors.

1.2 | Study conclusions and disease implications

In simple bivariate analyses, we confirmed our previous observations of increased severity of MRI-defined vascular brain injury markers among individuals characterized as having cognitive decline. The

largest effect-size difference between groups was for WMH distributed in posterior regions, which replicates earlier work in late-onset AD^{25,26} and in other genetic forms of AD.⁷ Similarly, we confirmed elevated cortical fibrillar amyloid levels by positron emission tomography (PET) among participants characterized as impaired. In univariate analyses of the plasma biomarker concentrations, neurofilament light chain (NfL) and plasma total tau (t-tau) were the only markers to differ between groups, similar to what has been reported previously in other cohorts.²⁷

Despite the modest differences in protein markers between groups in bivariate analyses, our analyses that used support vector machines to examine the association of protein markers simultaneously with neuroimaging markers suggest that both inflammation and AD/neurodegeneration are implicated in cerebrovascular disease, across lesion types (see Figure 1). Although different patterns of proteomic markers were associated with cerebrovascular markers and amyloid pathology, in all cases, both pro- and anti-inflammatory markers together with markers of AD/neurodegeneration contributed to the models, particularly among symptomatic individuals.

We interpret our results as preliminary evidence for biological interactions among vascular, inflammatory, and AD-specific processes in the evolution of clinical Alzheimer's symptoms in adults with DS that should stimulate future work on specific pathways. Visual inspection of Figure 1 shows a pattern where protein markers of general inflammation are relatively more involved with cerebrovascular disease among individuals without cognitive impairment, whereas markers reflective of AD and neurodegeneration are relatively more implicated in cerebrovascular disease among symptomatic individuals. Although it is not possible to infer causality, together with our previous observations, the findings suggest that inflammatory processes may give rise to cerebrovascular lesions in adults with DS early, which then interact with Alzheimer's pathology as symptoms emerge.^{21,22} Furthermore, cerebrovascular lesions, allowing for the leakage of proteins into the brain, may promote neuroinflammation and cerebrovascular disease.^{10,28–30} To this end, it is interesting to note that while some cerebrovascular disease marker values (eg., temporal lobe WMH) do not differ across cognitive severity groups, the pattern of proteomic biomarkers that is associated with them does; we interpret the findings as an indicator that although some cerebrovascular disease markers remain invariant across disease states, their underlying predictors may change.

Our analytic approach was based on prior work that used machine learning modalities to distinguish between cases (in those instances mild cognitive impairment-Down syndrome [MCI-DS] and DS-AD) and cognitively stable adults with DS.^{31–33} There are strengths and weaknesses with the application of support vector machines to probe associations between peripheral and central biological markers. One benefit is that this approach allows for the assessment of multiple markers simultaneously in multidimensional space, thereby resulting in higher order combinations of predictors, and in this case, plasma protein concentrations. Our results can therefore be interpreted as evidence that combinations of proteomic markers (such as those of inflammation

RESEARCH IN CONTEXT

1. Systematic Review: Adults with Down syndrome (DS) are at increased risk for developing Alzheimer's disease (AD). The study of the pathophysiology of AD among adults with DS is important because with increasing longevity, the public health impact of AD in this population is also increasing, and the knowledge gained may additionally inform our understanding of AD in the neurotypical population. There is debate about the role of cerebrovascular disease in AD. Previous work showed that magnetic resonance imaging (MRI) cerebrovascular disease markers are associated with AD risk and progression in both neurotypical and DS populations.
2. Interpretation: Here, we used an unbiased approach to demonstrate that inflammatory, vascular, and AD-related blood protein markers are associated with MRI measures of cerebrovascular disease in adults with DS.
3. Future Directions: Because individuals with DS have a low prevalence of classical vascular risk factors, our work, together with previous efforts, suggests that cerebrovascular disease is a core feature of AD that may be partially mediated by "endogenous" vascular and inflammatory mechanisms. Future work should examine these potential pathways.

HIGHLIGHTS

- Cerebrovascular disease is implicated in the clinical presentation and possibly pathogenesis of Alzheimer's disease (AD) among adults with Down syndrome (DS).
- Because individuals with DS do not exhibit the classical vascular risk factors that promote cerebrovascular disease, the cause of increased dementia-related cerebrovascular disease in DS is unknown.
- Support vector machine (SVM) analyses were used to explore the relationship of peripheral, blood-based proteomic neuroinflammatory, neuroimmunological, vascular, and AD-related biomarkers with radiological markers of cerebrovascular disease among adults with DS, classified as with and without Alzheimer's-related cognitive impairment.
- Inflammatory and neurodegeneration protein concentrations were associated with markers of cerebrovascular disease, particularly among individuals with symptoms of AD.

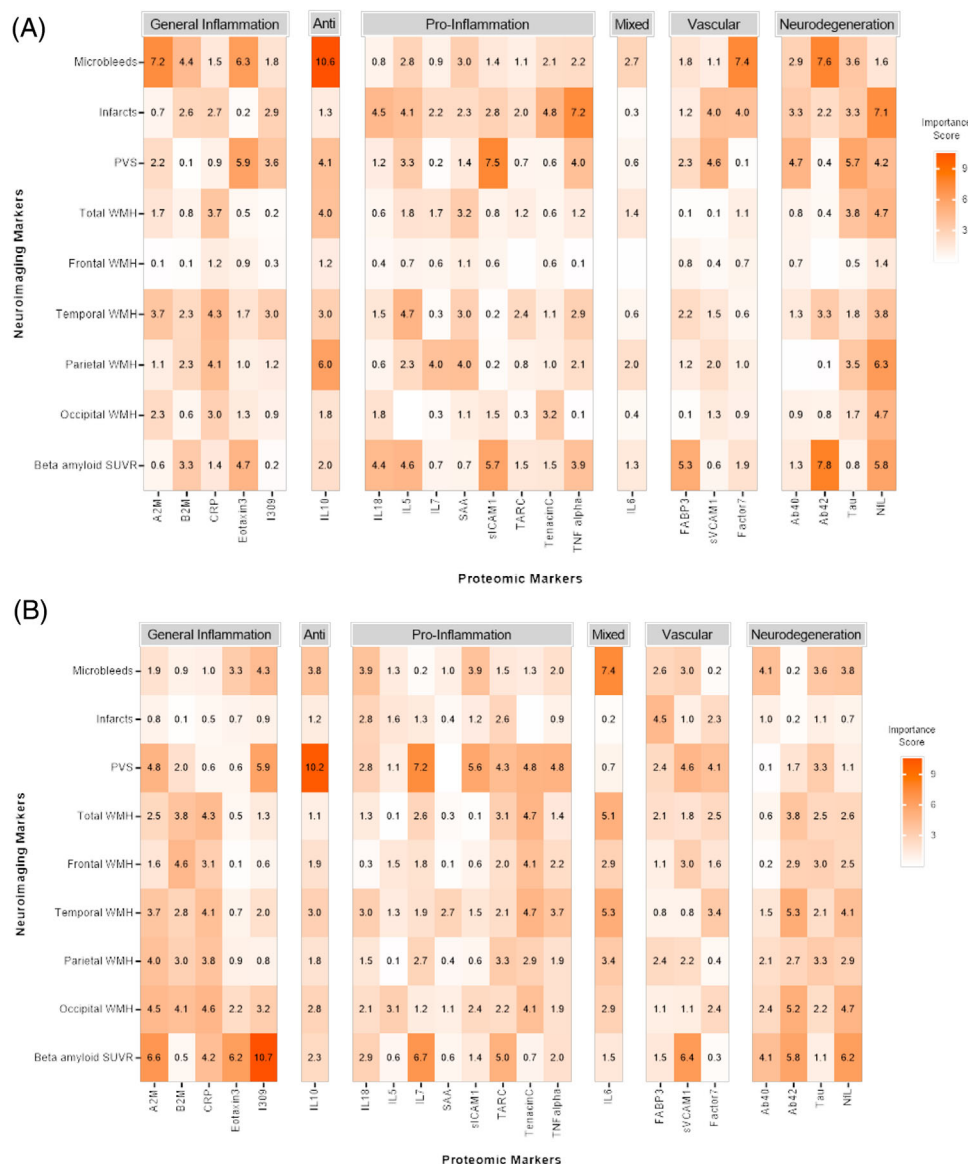


FIGURE 1 Summary of variable important plots. Each row in the heat map represents a separate analysis with numeric variable importance scores displayed and color coded (more saturated reds represent greater relative importance). Top figure (A) includes participants with cognitive impairment and the bottom figure (B) includes those with stable cognitive functioning. Protein markers are grouped by general function, including general inflammation, anti-inflammation, pro-inflammation, mixed anti-/pro-inflammation, vascular, and Alzheimer's disease/neurodegeneration. Anti, anti-inflammation; Mixed, mixed anti-/pro-inflammation

and AD pathology) are implicated in brain measures of cerebrovascular disease and Aβ as evidenced on neuroimaging. The more consistent associations observed in individuals in more progressed clinical states suggests that these proteomic markers may be involved with the clinical expression of AD and/or that they become manifest with disease progression. On the other hand, although the approach points to potential pathways to examine in follow-up experiments, the findings themselves cannot disambiguate specific pathways, directionality, or causality.

Observations derived from this study should stimulate additional research that examines mechanistic and causal inflammatory pathways, for example, as potential avenues to gain insight into the pathogenesis and potential intervention for the cerebrovascular disease that

is implicated in AD among people with DS or in the neurotypical population. Support vector machine approaches are typically used for diagnostic classification accuracy purposes³⁴ without regard to causality. In this vein, our analyses capture the extent to which proteomic and neuroimaging markers are related but they are agnostic to directionality. Therefore, from our analyses we cannot conclude whether higher or lower levels of plasma protein markers are related to greater or lesser degrees of brain disease. We also note that our proteomic assays included in this study are somewhat weighted toward inclusion of markers of inflammation, and as novel vascular biomarkers evolve, we may observe additional fluid biomarkers associated with cerebrovascular pathology, including those identified by efforts such as MarkVCID.³⁵

Our study motivates future work that should examine the emergence of cerebrovascular disease in the adult lifespan of adults with DS; causal relationships among cerebrovascular disease, inflammation, and AD pathology; and potential AD treatment, intervention, or prevention targets related to vascular and inflammatory disease in adults with DS. To this end, longitudinal studies that combine fluid biological markers, clinical characterization, neuroimaging data, and, ultimately, pathological data will be critical to continue this line of inquiry.

2 | CONSOLIDATED RESULTS AND STUDY DESIGN

Of the total DS participants enrolled in the ADDS study, 115 had available blood samples and MRI scans at the time of analysis and were included here. Cognitive diagnosis was determined through a consensus review process and included classification of cognitively stable, MCI-DS, and possible or definite AD dementia. MRI data were collected on a Siemens Prisma (Columbia University, MGH) or Philips Achieva (UC-Irvine) 3T platform. Vascular biomarkers were derived from MRI measures of WMH (total and regional), brain infarct, microbleeds, and enlarged PVS. A subset of participants with available blood samples and MRI data ($n = 84$) underwent amyloid PET imaging with Florbetapir and were also included. Proteomic assays were conducted across two platforms (Meso Scale Discovery and Quanterix) using electrochemiluminescence (ECL) techniques. A total of 500 μ L of plasma was used to assay proteomic markers spanning inflammation (general, pro- and anti-) and vascular factors as well as proteins linked to AD and neurodegeneration. Analyses were conducted stratified based on cognitive impairment status (nonimpaired [cognitively stable] and those considered symptomatic [MCI-DS, possible, or definite AD dementia]). Both t -tests and chi-square analyses were conducted to examine group differences in demographic, neuroimaging, and proteomic data. For the discrete variable analyses, we used support vector machine (SVM) to examine associations between neuroimaging and proteomic biomarkers.

Cognitively impaired participants were older, had higher levels of cortical amyloid, NfL, and had more severe measures of cerebrovascular disease relative to participants who were cognitively stable. Support vector machine proteomic panels produced high classification accuracy for individuals with cognitive impairment with at least one cerebral microbleed (area under the curve [AUC] = 1.00, sensitivity [SN] = 0.75, specificity [SP] = 1.00) and one or more infarct (AUC = 1.00, SN = 0.80, SP = 1.00). Although AUC remained high, the models showed reduced accuracy in their classification of those with stable cognition. Regarding other neuroimaging markers, the regression performance between the proteomic profile and cortical A β standardized uptake value ratio (SUVR), enlarged perivascular space severity, as well as WMH in the parietal and occipital lobes among those with cognitive impairment was high ($R^2 = 0.720$ to 0.789); however, the regression performance was much lower for total WMH as well as WMH in the frontal and temporal lobes ($R^2 = 0.376$ to 0.592). For participants who were cognitively stable, the regression performance for the proteomic profile was high for cortical A β SUVR, enlarged perivas-

cular space, and WMH in the temporal and occipital lobes ($R^2 = 0.708$ to 0.805) while much lower for WMH in the frontal and parietal lobes ($R^2 = 0.496$ to 0.577).

The heatmaps in Figure 1A, B show the relative importance of the different proteomic variables across the neuroimaging markers. There was an interesting pattern that emerged such that for participants with cognitive impairment, proteins linked to general inflammation and vascular and neurodegeneration had a higher relative importance on the variable importance plot for their association with microbleeds, infarcts, and enlarged perivascular space, whereas for those who were cognitively stable, these markers were more associated with A β SUVR and WMH.

3 | DETAILED METHODS AND RESULTS

3.1 | Methods

3.1.1 | Participants

We examined proteomic correlates of MRI-derived cerebrovascular disease markers in the same adults with DS we described in a previous report.⁹ Briefly, we included participants from ADDS with available MRI scans. Of the 138 participants who met this criterion, 115 had available blood samples for proteomic analysis. Participants were enrolled at Columbia University/New York State Institute for Basic Research in Developmental Disabilities, Massachusetts General Hospital, and University of California-Irvine. Participants and/or their legal guardians or representatives gave written informed consent for participation. All participants gave assent for each study procedure.

3.1.2 | Diagnostic assessment

Diagnostic procedures have been described in detail.³⁶ Briefly, a consensus panel, including clinician-researchers with expertise in assessment and diagnosis of dementia in adults with DS, reviewed neuropsychological, informant, and clinical data to assign one of four prevalent AD-related diagnoses: *cognitively stable*, indicating little evidence of significant cognitive decline; MCI-DS, indicating cognitive decline greater than expected for age but considered not sufficient for dementia; *possible AD dementia*, indicating substantial cognitive decline considered greater than MCI-DS; and *definite AD dementia*, indicating unambiguous evidence of clinically significant cognitive and functional decline.

3.1.3 | Neuroimaging

MRI data were collected on a Siemens Prisma (Columbia University, MGH) or Philips Achieva (UC-Irvine) 3T platform. Sequences included T1-weighted scan (repetition time [TR]/echo time [TE]/inversion time [TI]: 2300/2.96/900 ms; voxel size: $1 \times 1 \times 1$ mm³), T2-weighted fluid-attenuated inversion recovery (FLAIR; TR/TE/TI: 5000/386/1800 ms;

voxel size: $0.4 \times 0.4 \times 0.9 \text{ mm}^3$), and T2*-weighted gradient echo (GRE; TR/TE: 650/20 ms; voxel size: $0.8 \times 0.8 \times 4 \text{ mm}^3$) or susceptibility weighted image (SWI; TR/TE: 27/20 ms; voxel size: $0.9 \times 0.9 \times 1.5 \text{ mm}^3$).⁹

White matter hyperintensities

Total and regional WMHs were derived by applying a half Gaussian mixture model to intensity-normalized FLAIR images, summing the number of labeled voxels, and multiplying the sum by the voxel dimensions to yield volumes in cm^3 .³⁷ Outcome measures included total volume and volumes in frontal, temporal, parietal, and occipital lobes. We found previously that global WMH volume increased monotonically across diagnostic groups (CS < MCI-DS < possible AD dementia < definite AD dementia) and that this effect was strongest in the parietal lobes.⁹

Brain infarct

Brain infarcts were assessed visually on FLAIR and T1-weighted images. Infarcts were defined as hypointense lesions with diameter >5 mm, with hyperintense ring on FLAIR, and corresponding hypointense lesion on T1-weighted scans. The primary outcome was presence or absence of any infarcts. We reported that the frequency of infarcts was greater among adults with DS diagnosed with possible or definite AD dementia compared with the other groups.⁹

Microbleeds

Cerebral microbleeds were rated visually on GRE or SWI images.^{38–40} Microbleeds were identified as round hypointense lesions surrounded at least partially by parenchyma. Expert raters distinguished microbleeds from common mimics. We characterized participants as having 0 versus 1 or more detectable microbleed. In our previous study⁹ 43% of participants with definite AD dementia had evidence of at least one microbleed.

Enlarged PVS

T1-weighted and T2-weighted scans were used to rate the severity of enlarged PVS. The scale assigns a score of 0 (absent), 1 (1 to 3 observed enlarged PVS), or 2 to 13 brain regions to yield a total severity score that ranges from 0 to 26.^{41,42} Our previous study⁹ showed that enlarged perivascular space severity increased across AD diagnoses.

3.1.4 | Amyloid PET imaging

A subset of participants with available blood samples and MRI data ($n = 84$) underwent amyloid PET imaging with Florbetapir. Participants were scanned at Columbia University on a Siemens Biograph 64 system (voxel size = $1 \times 1 \times 2 \text{ mm}^3$, reconstruction = OSEM3D+TOF, $n = 10$); on a Siemens Biograph mMR system at MGH (voxel size = $2.1 \times 2.1 \times 2.0 \text{ mm}^3$, reconstruction = OP-OSEM, $n = 31$); and on a Siemens high-resolution research tomograph at UC-Irvine (voxel size = $1.2 \times 1.2 \times 1.2 \text{ mm}^3$, reconstruction = OP-OSEM3D, $n = 49$), following a standardized protocol (4 × 5 minutes frames; 50 to 70 min-

utes post-injection).⁴³ Anatomical data came from the application of FreeSurfer v.6.0⁴⁴ to the T1-weighted scans, which were co-registered to PET images to derive regional SUVRs with cerebellar cortex as reference. Our previous analyses⁹ showed elevated amyloid SUVR in all diagnostic groups relative to those characterized as cognitively stable.

3.1.5 | Proteomic assays

Plasma samples were analyzed at the University of North Texas Health Science Center Institute for Translational Research Biomarker Core with the Hamilton Robotic StarPlus. This system was used for both assay preparation and for aliquoting (as needed). Proteomic assays were commercially obtained from Quanterix and Meso Scale Discovery (MSD; www.mesoscale.com) and assayed according to previously published methods, using ECL.^{45,46} A total of 500 μL of plasma was used to assay the following markers, spanning general inflammatory proteins ($\alpha 2$ -macroglobulin [A2M], B2M, CRP, exotoxin 3, I-309), proinflammatory proteins (IL-18, IL-5, IL-6, IL-7, serum amyloid A [SAA], soluble intercellular adhesion molecule-1 [sICAM-1], TARC, tenascin C, tumor necrosis factor α [TNF- α]), an anti-inflammation protein (IL-10), vascular proteins (fatty acid binding protein 3 [FABP3], circulating vascular cell adhesion molecule-1 [sVCAM1], factor VII [Factor7]), as well as proteins linked to AD and neurodegeneration (A β 40, A β 42, t-tau, and NfL).

3.1.6 | Statistical analysis

Statistical analyses were conducted with R (v.3.3.3) statistical software (R Development Core Team, 2009). The general statistical approach examined whether a plasma proteomic panel was associated with cerebrovascular and amyloid PET neuroimaging markers. We stratified the analyses by cognitive impairment status, including impaired (cognitively stable) and those considered symptomatic (MCI-DS, possible, or definite AD dementia).

First, we conducted *t*-tests and chi-square analyses to examine group differences in demographic, neuroimaging, and proteomic data. Next, for the discrete variable analyses, we used support vector machines (or SVMs) to examine associations between neuroimaging and proteomic biomarkers. This classification method constructs hyperplanes in multidimensional space to allow separation of class labels to test the association of the proteomic markers with the neuroimaging outcomes. Classification accuracy for dichotomous neuroimaging outcomes (i.e., microbleeds, infarcts) was determined with receiver-operating characteristic (ROC) curves with a 5-fold internal cross-validation. SVM-based regression method (Support Vector Regression; SVR) was used to predict continuous outcome variables (i.e., regional WMH, amyloid PET SUVR). In SVR, R squared (R^2) is used as the primary regression performance metric. Variable importance plots were derived from the SVM/SVR analyses to examine the association of plasma proteomic proteins with neuroimaging biomarkers in models that were stratified by cognitive impairment status. Plasma

markers found to be higher on the variable importance plot reflected their higher relative impact on the model. To evaluate patterns of associations between proteomic and neuroimaging biomarkers, values from the variable importance plots were color-coded and plotted into heatmaps, which contained all outcomes together and protein markers grouped by type.

3.2 | Results

3.2.1 | Demographic information and unadjusted models

Table 1 displays demographic, neuroimaging, and plasma protein levels for included participants. Participants characterized as cognitively impaired were older but had similar sex distribution as those characterized as cognitively stable. As we reported previously,⁹ cognitively impaired individuals had elevated cortical amyloid levels and more severe measures of cerebrovascular disease than cognitively stable participants. These effects were most notable for parietal lobe WMH, enlarged PVS, and microbleeds. In univariate analyses, NfL and t-tau concentrations differed between groups, consistent with prior work.³²

3.2.2 | Associations of proteomic markers with neuroimaging outcomes

Figure 1 includes heatmaps that display the variable importance plots of each protein marker with each neuroimaging marker, stratified by cognitive status.

Cerebral microbleeds

The plasma proteomic panel produced a high detection accuracy (area under the curve [AUC] of 1.00) along with a sensitivity of 0.75 and specificity of 1.00 for classifying individuals as having at least one *cerebral microbleed* among those with cognitive impairment. The variable importance plot included a mix of inflammatory (IL-10, A2M, Eotaxin3, sICAM1, B2M) and AD/neurodegeneration (Aβ42, t-tau) markers (Figure 1A). For those with stable cognition, the plasma proteomic panel produced a similar higher level of detection accuracy (AUC = 1.00); however, sensitivity was lower at 0.26 compared with when it was applied to those with cognitive impairment, whereas specificity remained high at 1.00. As was seen among those with cognitive impairment, the top proteins in the variable importance plot reflected processes of inflammation (IL-6, I309, sICAM1, IL-18, IL-10) and AD/neurodegeneration (Aβ40, NfL, t-tau; Figure 1B).

Infarcts

Among participants with cognitive impairment, the proteomic panel accuracy for classifying individuals with one or more *infarct* was high (AUC = 1.00; sensitivity = 0.80; specificity = 1.00). The top variables in the variable importance plot included inflammatory (TNFα, Tenacin C, IL-18, IL-5), AD/neurodegeneration (NfL), and vascular (sVCAM1, Fac-

tor 7) specific markers (Figure 1A). The plasma proteomic panel did not accurately classify individuals with one or more infarct among those with stable cognitive functioning; despite a high AUC and specificity of 1.00 and sensitivity was 0.00. The top variables in the variable importance plot among those cognitively stable reflected primarily inflammatory (IL-18, TARC, IL-5) and vascular (FABP3, Factor 7) pathology (Figure 1B).

Perivascular spaces

There was a high regression performance ($R^2 = 0.789$) between the plasma proteomic profile and enlarged *perivascular space* severity. The top proteins in the variable importance plot included markers of inflammation (sICAM1, Eotaxin3) and AD/neurodegeneration (t-tau, Aβ40, NfL) for those with cognitive impairment (Figure 1A). In those with stable cognition, the regression performance was also high ($R^2 = 0.713$) with top proteins reflecting inflammatory processes (IL-10, IL-7, I309, sICAM1, TNFα; Figure 1B).

Total WMH

There was a moderate regression performance ($R^2 = 0.592$) for the plasma proteomic panel and *total WMH* among those with cognitive impairment. The top variables on the variable importance plot included markers of AD/neurodegeneration (NfL, t-tau) and inflammation (IL-10, CRP, SAA, IL-5; Figure 1A). A similar moderate regression performance was found among those with stable cognition ($R^2 = 0.666$) with primarily inflammatory markers (IL-6, Tenacin C, CRP, B2M) shown among the top variables in the variable importance plot (Figure 1B).

Frontal WMH. Among those with cognitive impairment, the regression performance between *frontal lobe WMH* and the plasma proteomic panel was low ($R^2 = 0.436$). Despite the low prediction performance, the top proteins on the variable importance plot included markers of Alzheimer's disease/neurodegeneration (NfL) and inflammation (IL-10, CRP, SAA, Eotaxin3; Figure 1A). The regression performance was also low for those with stable cognition ($R^2 = 0.496$). The top variables in the variable importance plot for those with stable cognition revealed strong drivers of inflammation (B2M, Tenacin C, CRP) followed by AD/neurodegeneration (t-tau, Aβ42; Figure 1B).

Temporal lobe WMH. There was a weak regression performance between plasma proteomic markers and *temporal lobe WMH* ($R^2 = 0.376$) among those with cognitive impairment. The top proteins in the variable importance plot reflected inflammation (IL-5, CRP, A2M) and markers of AD/neurodegeneration (NfL, Aβ42; Figure 1A). In contrast, there was a strong regression performance between the same plasma proteomic panel and temporal lobe WMH among those cognitively stable individuals ($R^2 = 0.733$), with the top variables in the variable importance plot reflecting similar elevations in markers of AD/neurodegeneration (Aβeta 42, NfL) and inflammation (IL-6, Tenacin C, CRP, TNFα; Figure 1B).

Parietal lobe WMH. There was a strong regression performance among those with cognitive impairment between the plasma proteomic

TABLE 1 Demographic and neuroimaging characteristics of participants characterized clinically as cognitively stable and impaired (MCI, possible AD dementia, definite AD dementia)

	Cognitively stable	Impaired	Statistic
N	78	36	–
Age, mean (SD) years	49 (6.4)	53 (6.2)	$t = 3.59, P < .001$
Sex, n (%) women	33 (42%)	12 (33%)	$\chi^2 = 0.49, P = .48$
Microbleed, n (%)	19 (24%)	16 (44%)	$\chi^2 = 3.77, P = .05$
Infarct, n (%)	11 (14%)	10 (28%)	$\chi^2 = 2.22, P = .13$
Perivascular space, mean (SD) severity, range	4.5 (3.9), 0–16	6.6 (4.7), 0–16	$t = 2.26, P = .02$
Total WMH volume, mean (SD) cm ³	2.1 (3.1)	3.1 (4.5)	$t = 1.20, P = .23$
Frontal WMH volume, mean (SD) cm ³	1.0 (2.1)	1.3 (3.6)	$t = 0.483, P = .63$
Parietal WMH volume, mean (SD) cm ³	0.30 (0.59)	0.86 (1.43)	$t = 2.25, P = .02$
Occipital WMH volume, mean (SD) cm ³	0.20 (0.38)	0.33 (0.55)	$t = 1.26, P = .21$
Temporal WMH volume, mean (SD) cm ³	0.21 (0.30)	0.26 (0.43)	$t = 0.59, P = .55$
A β cortical, mean (SD) SUVR	1.3 (0.21)	1.4 (0.30)	$t = 2.53, P = .01$
Plasma A2M, mean (SD)	1.2e + 09 (3.0e + 08)	1.2e + 09 (2.6e + 08)	$t = 0.32, P = .74$
Plasma B2M, mean (SD)	7688311 (2637578)	8436881 (2830684)	$t = 1.34, P = .18$
Plasma CRP, mean (SD)	1.3e + 07 (1.8e + 07)	9.7e + 06 (1.6e + 07)	$t = 0.99, P = .32$
Plasma Eotaxin3, mean (SD)	161 (1254)	105 (550)	$t = 0.33, P = .74$
Plasma FABP3, mean (SD)	3659 (1261)	3861 (1250)	$t = 0.79, P = .42$
Plasma Factor 7, mean (SD)	1250579 (302467)	1205275 (282978)	$t = 0.77, P = .44$
Plasma I309, mean (SD)	9.2 (5.7)	8.1 (4.7)	$t = 1.04, P = .30$
Plasma IL10, mean (SD)	0.78 (1.52)	0.50 (0.36)	$t = 1.54, P = .12$
Plasma IL18, mean (SD)	103 (41)	94 (40)	$t = 1.04, P = .29$
Plasma IL5, mean (SD)	0.30 (0.22)	0.31 (0.27)	$t = 0.10, P = .91$
Plasma IL6, mean (SD)	2.8 (10.7)	1.0 (0.8)	$t = 1.44, P = .15$
Plasma IL7, mean (SD)	5.1 (3.3)	5.1 (3.0)	$t = 0.09, P = .92$
Plasma amyloid beta 40, mean (SD)	448 (115)	465 (102)	$t = 0.79, P = .42$
Plasma amyloid beta 42, mean (SD)	15 (3.2)	16 (3.1)	$t = 1.00, P = .32$
Plasma total tau, mean (SD)	2.2 (0.73)	3.3 (1.65)	$t = 3.88, P < .001$
Plasma NfL, mean (SD)	19 (9.5)	34 (22.4)	$t = 3.79, P < .001$
Plasma PPY, mean (SD)	249 (437)	329 (440)	$t = 0.91, P = .36$
Plasma SAA, mean (SD)	3.1e + 07 (5.8e + 07)	4.5e + 07 (8.2e + 07)	$t = 0.90, P = .36$
Plasma sICAM1, mean (SD)	351971 (111246)	316430 (95090)	$t = 1.75, P = .08$
Plasma sVCAM1, mean (SD)	576982 (228304)	611023 (222528)	$t = 0.75, P = .45$
Plasma TARC, mean (SD)	251 (203)	216 (145)	$t = 1.04, P = .30$
Plasma Tenascin-C, mean (SD)	69223 (15352)	75167 (24003)	$t = 1.36, P = .17$
Plasma TNF- α , mean (SD)	2.9 (0.87)	2.9 (0.90)	$t = 0.09, P = .92$
Plasma TPO, mean (SD)	659 (518)	1440 (4106)	$t = 1.13, P = .263$

Note that sample size for amyloid PET analysis was $n = 84$. Note too that each MRI measure had slightly different sample size because of poor scan quality due to motion or truncated scan acquisition: microbleed ($n = 99$), WMH ($n = 109$), infarct ($n = 113$), perivascular space ($n = 110$).

Abbreviation: WMH, white matter hyperintensity; WMH, white matter hyperintensities; SUVR, standardized uptake volume ratio; A2M, α 2-macroglobulin, B2M, β 2-macroglobulin; CRP, c-reactive protein; FABP3, fatty acid binding protein 3; IL, interleukin; NfL, neurofilament light chain; PPY, pancreatic polypeptide; SAA, serum amyloid A; sICAM1, soluble intercellular adhesion molecule-1; sVCAM1, soluble vascular cell adhesion molecule-1; TARC, thymus and activation regulated chemokine; TNF- α , tumor necrosis factor α ; TPO, thrombopoietin.

profile and *parietal lobe WMH* ($R^2 = 0.770$). The variable importance plot revealed the top two markers as NfL and IL-10 followed less closely by additional markers of inflammation (CRP, SAA, IL-7; Figure 1A). The regression performance among those with stable cognitive functioning was ($R^2 = 0.577$). The top variables as shown on the variable importance plot were related to inflammation (A2M, CRP, IL-6, TARC, B2M) and Alzheimer's disease/neurodegeneration (t-tau, NfL; Figure 1B).

Occipital lobe WMH. In those with cognitive impairment, there was a strong regression performance between the plasma proteomic profiles and *occipital lobe WMH* ($R^2 = 0.720$). The driving proteomic marker, as illustrated in the variable importance plot, was NfL, a marker of neurodegeneration, followed by several markers of inflammation (Tenacin C, CRP, A2M, IL-18; Figure 1A). Among individuals with stable cognition, the regression performance was also high ($R^2 = 0.708$). The top variables in the variable importance plot included markers of neurodegeneration (A β 42, NfL) and inflammation (CRP, A2M, B2M, Tenacin C; Figure 1B).

Cortical A β SUVR

Among those with cognitive impairment, there was a strong regression performance between the plasma proteomic panel and *amyloid beta cortical SUVR* ($R^2 = 0.776$) with the top variables including markers of neurodegeneration (A β 42, NfL) and inflammation (sICAM1, Eotaxin3, IL-5, IL-18; Figure 1A). For cognitively stable cases, the regression performance was also high ($R^2 = 0.805$) with the top variables in the variable importance plot reflecting a combination of inflammatory (I309, IL-7, A2M), vascular (sVCAM1), and neurodegeneration (A β 42, NfL; Figure 1B).

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