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RESEARCH ARTICLE

Plasma protein risk scores for mild cognitive impairment and Alzheimer's disease in the Framingham heart study

Habbiburr Rehman[1] | **Ting Fang Alvin Ang**[2,3] | **Qiushan Tao**[4] | **Rhoda Au**[1,2,3,5,6,7] | **Lindsay A. Farrer**[1,3,5,6,7,8,9] | **Wei Qiao Qiu**[4,7,10] | **Xiaoling Zhang**[1,6,9] | **for the Alzheimer's Disease Neuroimaging Initiative**

[1]Departments of Medicine (Biomedical Genetics), Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts, USA

[2]Departments of Anatomy & Neurobiology, Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts, USA

[3]Departments of Neurology, Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts, USA

[4]Departments of Pharmacology & Experimental Therapeutics, Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts, USA

[5]Departments of Epidemiology, Boston University School of Public Health, Boston, Massachusetts, USA

[6]Framingham Heart Study, Boston University School of Medicine, Framingham, Massachusetts, USA

[7]Alzheimer's Disease Research Center, Boston University School of Medicine, Boston, Massachusetts, USA

[8]Departments of Ophthalmology, Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts, USA

[9]Departments of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA

[10]Departments of Psychiatry, Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts, USA

Correspondence
Xiaoling Zhang, Boston University Chobanian & Avedisian School of Medicine, Boston, MA, USA, 72 East Concord Street, E223, Boston, MA 02118, USA.
Email: zhangxl@bu.edu

Wendy Wei Qiao Qiu, Boston University Chobanian & Avedisian School of Medicine, 72 East Concord Street, R-623D, Boston, MA 02118, USA.
Email: wqiu67@bu.edu

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Abstract

INTRODUCTION: It is unclear whether aggregated plasma protein risk scores (PPRSs) could be useful in predicting the risks of mild cognitive impairment (MCI) and Alzheimer's disease (AD).

METHODS: The Cox proportional hazard model with the Least Absolute Shrinkage and Selection Operator penalty was used to build the PPRSs for MCI and AD in 1515 Framingham Heart Study Generation 2 with 1128 proteins measured in plasma at exam 5 (cognitively normal [CN] = 1258, MCI = 129, AD = 128).

RESULTS: MCI PPRS had a hazard ratio (HR) of 6.97 [5.34, 9.12], with a discriminating power (C-index = 82.52%). AD PPRS had a HR of 5.74 [4.67, 7.05] (C-index = 88.15%). Both PPRSs were also significantly associated with cognitive changes, brain atrophy, and plasma AD biomarkers. Proteins in the MCI and AD PPRSs were involved in several pathways related to leukocyte, chemotaxis, immunity, inflammation, and cellular migration.

DISCUSSION: This study suggests that PPRSs serve well to predict the risk of developing MCI and AD as well as cognitive changes and AD-related pathogenesis in the brain.

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KEYWORDS
Alzheimer's disease, brain volume, mild cognitive impairment, plasma protein risk score, p-tau181

Highlights

- PPRSs were developed for the risk of AD and AD preclinical stage, MCI.
- PPRSs were developed for MCI and AD associated with cognitive changes, loss of brain volume, and increasing level of plasma AD biomarkers.
- Leukocyte, chemotaxis, immunity, inflammation, and cellular migration enriched in proteins were identified as being involved in MCI and AD PPRSs.

1 | BACKGROUND

Alzheimer's disease (AD) is a long-term degenerative process defined by initial memory impairment and cognitive decline that can eventually affect behavior, speech, visuospatial orientation, and motor function, accounting for up to 80% of all dementia cases.[1,2] AD pathological changes begin during a preclinical phase, often years before clinical symptoms appear, with the accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau.[3,4] It has been reported that the number of AD patients worldwide is about 44 million and projected that this number could triple by 2050 due to the aging population.[4–6] AD develops in three clinically distinct stages: cognitively unimpaired, prodromal signs of mild cognitive impairment (MCI), and onset of dementia.[2,7–10] A robust antemortem diagnosis of AD considers results from a detailed neuropsychological test battery, neurological and brain imaging examinations, and often measurement of ATN (amyloid/tau/neurodegeneration) biomarkers (e.g., Aβ40, Aβ42, phosphorylated tau (p-tau) isoforms, and total tau (t-tau)/neurofilament light protein (NFL).[8,11] However, there are no reliable biomarkers for predicting and monitoring the incidence of MCI and MCI to AD progression.

Cerebrospinal fluid (CSF) Aβ and tau, structural MRI for measurement of brain volume, (18)F-2-fluoro-2-deoxy-D-glucose ([(18)F]FDG) positron emission tomography [PET] for measurement of brain metabolism, and amyloid-PET for quantification of insoluble Aβ deposits were recognized as valid tools for AD diagnosis.[4,12–14] Remarkably, loss of hippocampal volume on MRI and CSF Aβ42 to Aβ40 ratio, t-tau, and p-tau are predictive of longitudinal changes in cognitive assessment in the context of rising AD pathology and its clinical consequences.[15–18] These biomarkers accurately distinguish AD from cognitively normal (CN) individuals with a mean sensitivity of 80% and specificity of 82% for Aβ42, sensitivity of 82% and specificity of 90% for t-tau, and sensitivity of 80% and specificity of 83% for p-tau.[2] A recent study reported that the 48 CSF protein panel outperformed existing ATN biomarkers in predicting the likelihood of AD and related outcomes, as well as cognitive changes.[19] CSF biomarkers for AD have been shown to predict progression to AD dementia from MCI with more than 80% accuracy.[18,20,21] Combination of CSF biomarkers with structural or functional brain imaging markers may provide higher diagnostic accuracy than the CSF biomarkers or imaging biomarkers alone.[22] Biomarkers for predicting MCI incidence are still evolving. Neuropathologic examination of older subjects who died with a clinical diagnosis of CN or MCI often revealed pathological markers similar to those with AD.[23] The use and testing of ATN biomarkers may be restricted to certain specialist and academic centers due to limitations such as apprehension about using a perceived invasive

procedure like lumbar puncture, lack of familiarity with test result analysis, and doubt about the medical importance of knowing an individual's biomarker status, as well as low acceptance of lumbar puncture from patients.[2,8,24,25] Therefore, it is critical to develop biomarkers for MCI or early preclinical stages of AD that are inexpensive and may be routinely utilized to promote early intervention and delay disease progression or prevent the onset of AD dementia.[17]

Recent trends indicate that biomarkers for diagnosis of AD continuum shifting to plasma/blood-based biomarkers because they are relatively common biological samples in medical and research settings, and venipuncture is safe, invasive, and inexpensive in comparison to lumbar puncture and imaging.[2,4,26] Specifically, plasma p-tau is an emerging biomarker for AD diagnosis and prediction.[4,12,27,28] Although several other studies have also shown that other protein markers in blood distinct from Aβ and tau also performed well in AD classification and prediction,[7,29–32] they do not outperform the CSF biomarkers for AD. One recent study identified 32 dementia-associated plasma proteins using a large-scale proteomics that were involved in proteostasis, immunity, synaptic function, and extracellular matrix organization.[33] However, it is unclear whether aggregated plasma protein risk score (PPRS)[34] derived from a single sample could be useful for the identification of the risk of AD. In addition, currently, there are no blood biomarkers for incident MCI, which is the critical stage for the prevention of and intervention in AD. This study aims to investigate the association between PPRS and the risk of MCI, AD incidence, and related outcomes.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using PubMed sources. Several studies in the literature found that plasma/blood proteins including ATN as well as proteins other than ATN can be used to predict the risk of AD. However, recent advances in large-scale proteomics measurement techniques have enabled the discovery of novel protein biomarkers. While the aggregated protein risk score (PPRS) derived from a single plasma sample may be a viable biomarker for AD, the preclinical stage MCI must be investigated. The essential references are properly cited.
2. **Interpretation**: Our findings show that MCI and AD PPRSs derived from proteins that differ from ATN biomarkers can be used to predict the probability of developing MCI and AD incidences, respectively. In addition, both MCI and AD PPRSs are associated with cognitive changes, brain volume loss, and an increased level of plasma AD biomarkers. Proteins in MCI and AD PPRSs were shown to be enriched in various leukocyte, chemotaxis, immunity, inflammation, and cellular migration pathways.
3. **Future directions**: Development of PPRSs for biological AD based on CSF and imaging ATN biomarker categorization may be considered as potential future efforts. Combining proteins involved in MCI and AD PPRSs with plasma ATN biomarkers may improve the ability to predict the likelihood of AD and related outcomes.

2 | METHODS

2.1 | Data source

Data for this study were obtained from Offspring (Generation 2) cohort participants of the Framingham Heart Study (FHS), a single-site, multigeneration, community-based, prospective cohort study of health in Framingham, Massachusetts. We included participants with available aptamer-based SOMAscan proteomics assay measurements (n = 1913) who were rigorously evaluated for cognitive function and followed longitudinally until February 2024 (Figure S1). The design and selection criteria of the FHS participants were described previously.[35] A total of 398 individuals were excluded due to missing education years (n = 253), missing apolipopotein E (APOE) ε4genotype (n = 84), other type of dementia (n = 47), and missing follow-up years (n = 14). A total of 1515 participants remained for the primary analyses. Informed consent was obtained from all study participants, and the Institutional Review Board of Boston University approved the study protocol. For external validation of our findings, we used CSF SOMAscan proteomics data from Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.[36] More details about ADNI are available at (http://adni.loni.usc.edu/). Also, the FHS participant with missing education years and APOE ε4 genotype (total n = 321, CN = 276, MCI = 21, AD = 24) were used for the internal testing of the results. These 321 participants were not included as part of the training data.

2.2 | Cognitive assessment

Surveillance of cognitive impairment and incident dementia in the Offspring cohort began in 1979, at the second health exam, when the group was relatively young on an average (mean[range]) 44 [17 to 77] years old, to develop a dementia-free cohort. At the beginning of the fifth health exam (1991 to 1995), the Mini-Mental State Examination (MMSE),[37] and Montreal Cognitive Assessment (MoCA) were used to monitor changes in cognitive state. A decrease in MMSE performance of three or more points from the previous assessment, or five or more points overall, and a MoCA score below 23 would indicate a cognitive status change that required investigation by a dementia diagnostic panel composed of at least one neurologist and one neuropsychologist. Furthermore, from 1999 to 2005, all surviving Generation 2 individuals were invited to an in-depth cognitive evaluation, which included screening for incident cognitive impairment.[38] The panel determines whether a person had dementia, dementia subtype, and the date of onset, using data from previously performed sequential neurologic and neuropsychological examinations, telephone interviews with CloseKnit medical records, and neuroimaging studies. The diagnosis

of dementia was established using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria,[39] and AD was diagnosed based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[40] MCI without dementia was defined during dementia monitoring as a person who does not progress to dementia but may suffer decline but never go beyond MCI. Further, the MCI stage was characterized (e.g., amnestic, non-amnestic, specific cognitive domains affected).

Cognitive factor scores for memory, language, and executive function domains were determined. Scores were co-calibrated to ensure they were on the same scale regardless of the cognitive battery used. An expert panel of neuropsychologists and a behavioral neurologist classified each neuropsychological test item into one of three domains. Cognitive scores with standard error > 0.6 or derived solely from MMSE, which has a ceiling effect, were excluded.[41] Cognitive factor scores close to exam 5 and MMSE score (exams 5 to 8) were used for association analysis with MCI and PPRSs.

2.3 | Proteomic profiling lab assay

The aptamer-based SOMAscan proteomics platform was utilized to assay 1373 plasma proteins in two batches (batch 1: $n = 821$ and batch 2: $n = 1092$) from participants who attended exam 5. SOMAscan uses chemically modified single-stranded DNA aptamers to assess proteins in an accurate and high-throughput approach.[42,43] Each sample was multiplied by its allocated scale factor. Median normalization was employed to reduce sample or assay biases induced by differences in total protein concentration between samples, pipetting variance, reagent concentration variation, assay timing, and other sources of systematic variability within a single plate run. A total of 1128 proteins remained for analysis after excluding 245 proteins due to many missing observations ($n = 821$, approximately 43%) and a natural logarithmic transformation was employed to achieve a normal distribution for each protein.

2.4 | Brain imaging

Brain MRI examinations began in 1999 at the FHS, and although most participants had multiple MRI examinations, we included the measurement closest to exam 5 at which blood specimens were obtained for plasma proteomic assays. Procedures for acquiring images and deriving have been described in detail elsewhere.[44,45] In brief, a Siemens 1-T MRI machine (Siemens Medical) with a T2-weighted double spin-echo coronal imaging sequence was used. A central laboratory blinded to demographic and clinical information processed and quantified the digital information on brain pictures using a custom-written computer application running on a UNIX Solaris platform (Sun Microsystems). Semiautomated pixel distribution analysis was used to compute brain volume by mathematically modeling MRI pixel intensity histograms for CSF and brain matter (white matter and gray matter) to establish the ideal pixel intensity threshold for distinguishing CSF from brain matter. The semiautomated segmentation methodology for measuring total cranial volume, total cerebral brain volume, frontal lobar brain volume, parietal lobe brain volume, temporal lobe brain volume, and hippocampus volume, as well as their inter-rater reliability, was previously reported.[46,47] Furthermore, each analyst was thoroughly instructed in how to maintain stringent precision, with intraclass (analyst) coefficients reaching 90% across the board. All brain volumes are expressed as percentages of intracranial volume to adjust for head size. In this study, we applied logit transformation to remove skewness.

2.5 | Plasma AD biomarkers

Plasma AD biomarkers (p-tau181, t-tau, Aβ40, and Aβ42) were measured at different exams (9, 8, and 7) in the FHS Offspring participants using blood samples taken after several years of blood samples that were used for protein measurements (i.e., exam 5). The Quanterix Simoa Assay 2.0 kit was used to measure plasma biomarkers from an EDTA plasma sample.[48] Quanterix has developed an approach to detecting thousands of single protein molecules simultaneously. Utilizing the same reagents as a conventional ELISA, this method has been used to measure proteins in a variety of different matrices (e.g., serum, plasma, cerebral spinal fluid, urine, cell extracts) at femtomolar (fg/mL) concentrations, offering a roughly 1000-fold improvement in sensitivity. Samples arrived on dry ice and were stored at −80°C upon arrival. Before analysis, samples were thawed completely at room temperature (requiring approximately 30 to 60 min, depending on the volumes provided), and mixed thoroughly until visibly homogeneous via gentle inverting 10 times. For a detailed description, see sMethod. Before analysis, extreme outlier samples were removed, then data were normalized and log-transformed.

2.6 | Statistical analyses

We compared baseline characteristics across diagnosis groups (CN = 1258, MCI [amnestic] = 129 [71], and AD = 128) using one-way ANOVA for continuous data and Pearson's chi-squared test for categorical variables. A Cox proportional hazard (PH) model was used to analyze the association of plasma proteins with the incidence of MCI and AD. The Least Absolute Shrinkage and Selection Operator (LASSO) penalization method[49,50] was applied to determine the number of proteins to be included in the calculation of the PPRS (see sMethod, Tables S1 and S2, and Figures S2, S3, and S4 in supplemental material for detailed information). Hazard ratios (HRs) and their 95% confidence limits were estimated using Cox PH models to assess the effect of MCI and AD PPRSs on the incidence of MCI and AD, respectively. We tested four models each with the following terms: (1) MCI/AD PPRS only; (2) MCI/AD PPRS, age, sex, and years of education; (3) terms in Model 2 + APOEE ε4 carrier status; and (4) a reference model including covariates only (age, sex, education of years, and APOEE ε4 carrier status). All individuals were classified as low, middle, or high MCI and

AD PPRs based on tertials. The discriminating power of each model was quantified using the concordance index (C-index). In addition, we also used FHS cardiovascular disease (CVD) risk score as an additional confounding risk factor to test its effect on the performance of MCI and AD PPRSs. FHS CVD risk score derived by several risk components for CVD, such as cholesterol, diabetes, smoking, blood pressure, and age.[51]

The association of MCI and AD PPRSs with cognitive domains (memory, language, and executive function [CN = 736, MCI = 103, and AD = 93] and MMSE [CN = 914, MCI = 113, and AD = 87], several brain MRI traits [CN = 809, MCI = 100, and AD = 78] including volume measures (hippocampal, total brain, temporal lobe, parietal lobe, and ventricles), total gray and white matter, total CSF, and plasma AD biomarkers (p-tau181 [CN = 736, MCI = 65, and AD = 42], t-tau [CN = 725, MCI = 70, and AD = 41], and Aβ40 and Aβ42 [CN = 1075, MCI = 119, and AD = 112]) was tested (Table S3). The average time between protein and MRI measurements was 8.29 years (Table S3). We compared the distributions of cognitive domains, brain MRI traits, and plasma AD biomarkers among individuals grouped into low, medium, and high MCI and AD PPRS levels using ANOVA and *t*-test. All statistical analyses were carried out with R software version 4.3.1; hypothesis tests were two-sided, and *p* values <0.05 were considered statistically significant.

2.7 | Gene Ontology enrichment analysis

We utilized the ClusterProfiler package in R to determine the over-represented significant Gene Ontology (GO) biological process pathways in the resultant proteins in both MCI and AD PPRSs using hypergeometric tests with all human coding genes/proteins as background/reference.[52,53] To remove redundant pathways/terms with 70% and more similarity, the "simplify ()" with 0.7 cutoff was used, and a false discovery rate (FDR) <0.05 was considered a significant threshold.

3 | RESULTS

3.1 | Participant characteristics

Participants who did not have MCI or dementia were included (n = 1515). As expected, longitudinal cognitive status was significantly associated with age (p = 9.16e-45), years of education (p = 5.77e-4), sex (p = 1.46e-4), and APOE ε4 (p = 0.002) (Table 1). AD participants were older, more likely female and APOE ε4 carriers, and less educated compared to CN and MCI participants. The average follow-up period was similar for MCI (18.10 ± 6.00) years and AD cases (17.09 ± 6.35) years, although approximately 6 and 7 years longer than for CN (p = 1.23e-35 Table 1). CN individuals had longer follow-up times since they were followed until death, or they were censored for MCI and AD at their last dementia surveillance.

3.2 | Association of plasma protein risk score with incidence of MCI and AD

We derived a PPRS for developing MCI comprising 36 proteins and a PPRS for AD risk comprising 50 proteins (Tables S1 and S2 and Figures S2, S3, and S4) of which five proteins are common to both PRSSs. The MCI PPRS was significantly associated with MCI incidence (Model 1: HR = 6.97 [5.34, 9.12], p = 6.7e-46). This finding remained significant after adjusting for age, sex, and education (Model 2: HR = 5.20 [3.82, 7.08], p = 1.5e-25) and not altered by further adjustment for APOE ε4 status (Model 3: HR = 5.22 [3.83, 7.12], p = 1.4e-25) (Table 2). The C-indexes for MCI PPRS were for Model 1 (82.52%), Model 2 (84.61%), and Model 3 (84.8%). The MCI PPRS model performed significantly better than the reference model, that is, without PPRS (C-index = 78.8%) (Table 2). Similarly, the AD PPRS was significantly associated with AD incidence (Model 1: HR = 5.74 [4.67, 7.05], p = 5.6e-62), including in models incorporating covariates (Model 2: HR = 3.70 [2.83, 4.85], p = 2.1e-21; and Model 3: HR = 3.67 [2.79, 4.82], p = 1.7e-20). The C-index for AD PPRS in Model 1 was 88.15%, similar to the value for the reference model (88.73%). However, AD PPRS prediction improved when adding covariates. C-indexes for Models 2 and 3 were 90.64% and 91.28%, respectively (Table 2). In addition, as shown in the reference model, APOE ε4 genotype is a strong predictor of AD risk (HR = 2.92 [2.01, 4.25], p = 1.9e-8) but not associated with MCI incidence (p = .14) (Table 2). Performance of MCI and AD PPRSs remain unaltered after adjusting for CVD risk score as an additional confounding factor in Model 3 (Table S4). The association and importance of each protein with MCI and AD incidences are given in Figures S3 and S4, respectively.

Survival analysis revealed that individuals with a high MCI PPRS had a substantially higher probability of developing MCI compared to those with a medium or low PPPRS (p = 6.6e-34, Figure 1A). A similar pattern was observed for the AD PPRS and the probability of developing AD (p = 8.2e-56, Figure 1B). After adjusting for age years (60, 70, and 80 years), the probability of experiencing an incidence of MCI and AD increased several times when the PPRSs for MCI and AD were high, respectively, especially in old age groups (Figure S5).

3.3 | MCI and AD PPRSs negatively correlated with cognitive changes

Next, we examined the relationship between MCI and AD PPRSs and cognitive domains (memory, language, and executive function factor scores) as well as global cognitive function (MMSE) score. MCI PPRS was significantly negatively correlated with memory (R = –0.29, p < 2.2e-16), language (R = –0.18, p = 7.1e-8), executive function (R = –0.31, p < 2.2e-16), and baseline MMSE score (R = –0.18, p = 2.5e-9) (Figure 2A). Cognitive factors gradually declined as AD PPRS increased (memory: R = –0.3, p < 2.2e-16; language: R = –0.32, p < 2.2e-16; and executive function: R = –0.39, p < 2.2e-16);

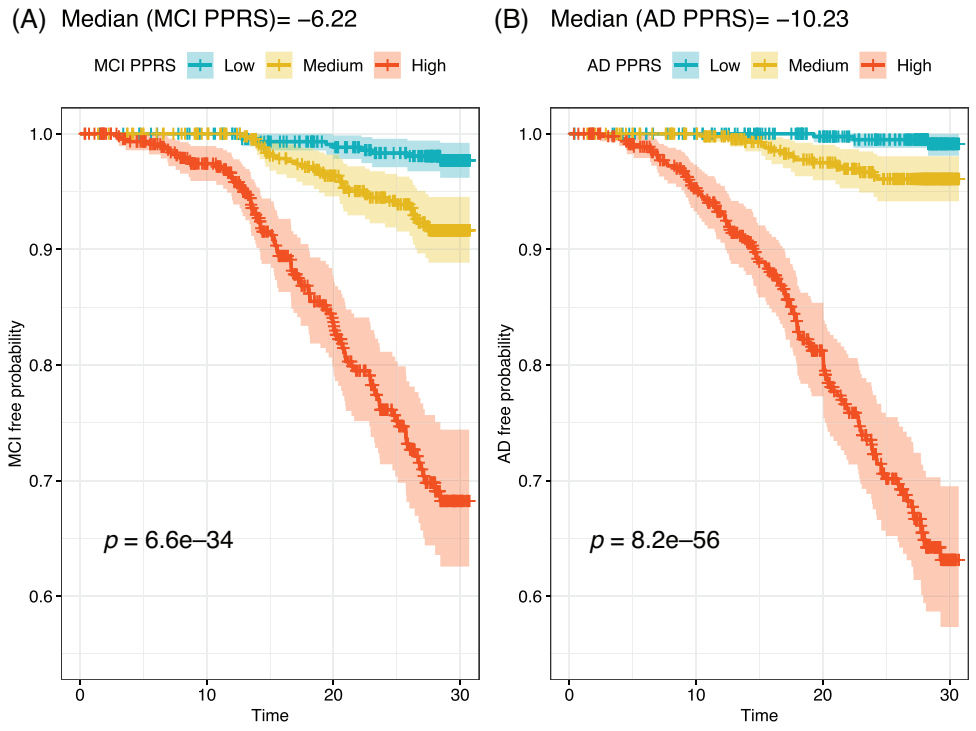

FIGURE 1 Kaplan–Meier analysis for (A) MCI-free probability based on different levels (low, medium, and high) of MCI PPRS and (B) AD-free probability based on different levels (low, medium, and high) of AD PPRS. MCI, mild cognitive impairment; AD, Alzheimer's disease; PPRS, plasma protein risk score.

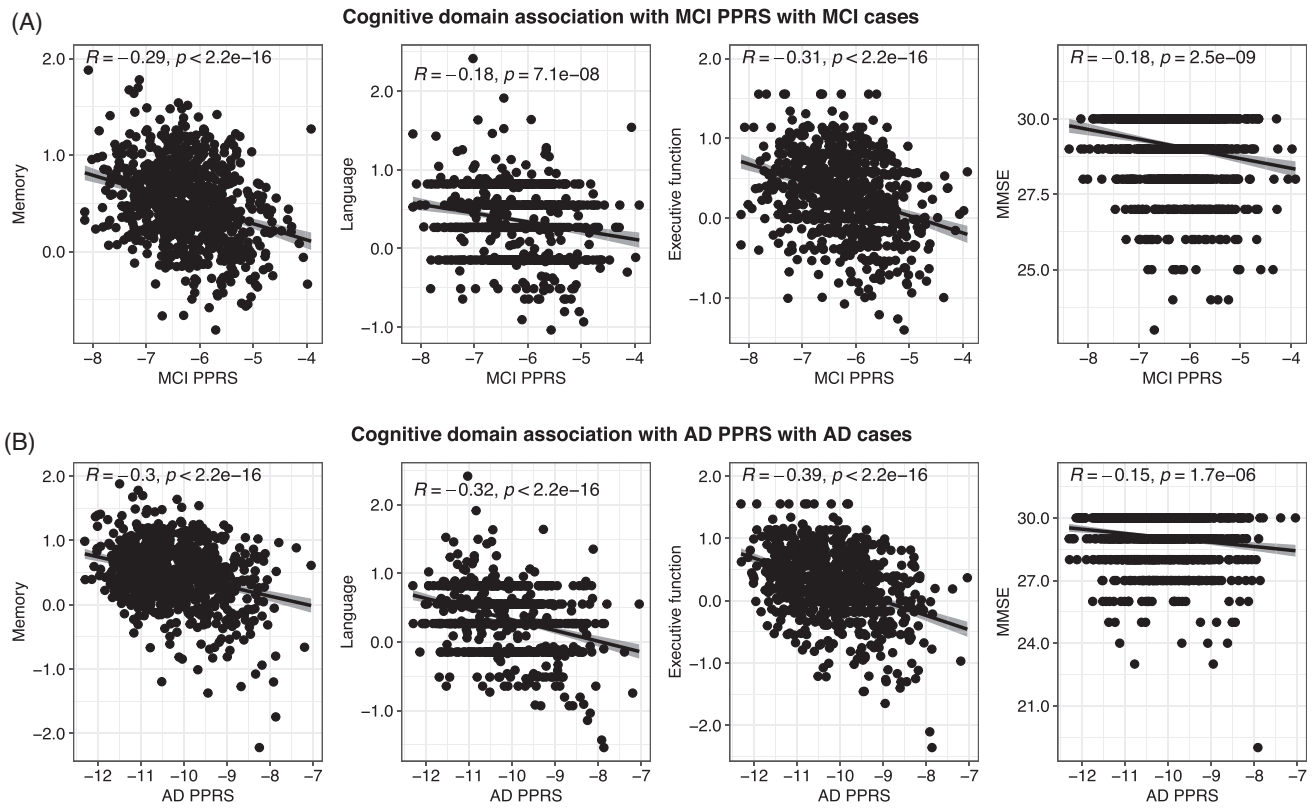

FIGURE 2 Cognitive domains association with (A) MCI PPRS and (B) AD PPRS. MCI, mild cognitive impairment; AD, Alzheimer's disease; PPRS, plasma protein risk score; MMSE, Mini-Mental State Examination. Memory, language, and executive function measurements were closest to exam 5 and MMSE was from exam 5 (baseline).

TABLE 1 Baseline characteristics of known risk factors in FHS Offspring participants.

Characteristic[a]	Overall, $n = 1515$[a]	CN, $n = 1258$	MCI (amnestic), $n = 129$ (71)	AD, $n = 128$	p[b]
Age	55.05 (9.85)	53.53 (9.60)	60.47 (7.80)	64.62 (6.13)	9.16e-45
Education years	14.06 (2.60)	14.17 (2.56)	13.71 (2.70)	13.33 (2.74)	5.77e-04
Sex					1.46e-04
Male	686 (45.28)	584 (46.42)	66 (51.16)	36 (28.13)	
Female	829 (54.72)	674 (53.58)	63 (48.84)	92 (71.88)	
APOE ε4					0.002
0	1,180 (77.89)	997 (79.25)	99 (76.74)	84 (65.63)	
1	335 (22.11)	261 (20.75)	30 (23.26)	44 (34.38)	
Follow-up years	23.03 (7.77)	24.14 (7.59)	18.10 (6.00)	17.09 (6.35)	1.23e-35

Abbreviations: AD, Alzheimer's disease; CN, cognitively normal; MCI, mild cognitive impairment.
[a]Mean (SD); *n* (%).
[b]One-way ANOVA; Pearson's chi-squared test.

also, MMSE at baseline was significantly correlated with AD PPRS ($R = -0.15$, $p = 1.7e\text{-}6$) (Figure 2B). MMSE showed a more negative correlation with MCI PPRS at exam 5 (baseline) and exam 6, while with AD PPRS at exams 7 and 8 (Figure 2 and Figure S6). Individuals with high MCI and AD PPRSs had significantly lower MMSE and cognitive domain scores as they aged compared to those with low MCI and AD PPRSs (Figures S7 and S8).

3.4 | Loss of brain volume significantly associated with higher plasma protein risk score

Hippocampal volume, temporal and parietal lobe volumes, and total gray and white matter were progressively smaller, whereas total CSF and third ventricle volume progressively increased, from low to high MCI PPRS ($p < 0.001$ for all comparisons) (Figure 3A). Most of these patterns were evident when individuals were stratified by tertiles of the AD PPRS ($p < 0.001$ for all comparisons) (Figure 3B). These findings were largely attenuated, but most remained statistically significant among individuals younger than 60 years (Figure S9) and among CN participants (Figure S10), indicating a tendency for decreased hippocampal and temporal lobe volumes among individuals with a high MCI or AD PPRS even before disease symptoms appeared.

3.5 | Higher plasma protein risk score associated with increasing plasma AD biomarkers

Level of plasma AD biomarker is significantly increased in individuals with high MCI PPRS (p-tau181: $p = 0.0013$ [low vs medium], $p = 3.7e\text{-}7$ [low vs high], $p = 0.011$ [medium vs high]; t-tau: $p = 0.0088$ [low vs high]; Aβ40: $p = 0.00039$ [low vs medium], $p = 2.6e\text{-}5$ [low vs high]; Aβ42: $p = 0.0047$ [low vs high] Figure 4A) and AD PPRS (p-tau181: $p = 0.00034$ [low vs medium], $p = 5.2e\text{-}10$ [low vs high], $p = 0.00067$ [medium vs high]; t-tau: $p = 1e\text{-}5$ [low vs high], $p = 0.0024$ [medium vs high]; Aβ40: $p = 0.0072$ [low vs medium], $p = 3.3e\text{-}5$ [low vs high]; Aβ42: $p = 0.0028$ [low vs medium], $p = 0.033$ [low vs high] Figure 4B). These associations were also significant in individuals younger than 60 years for the comparisons among MCI PPRS groups (p-tau181: $p = 0.037$ [low vs medium], $p = 0.00047$ [low vs high], $p = 0.042$ [medium vs high]; Aβ40: $p = 0.028$ [low vs medium], $p = 4.4e\text{-}5$ [low vs high], $p = 0.029$ [medium vs high]; Aβ42: $p = 0.007$ [low vs medium], $p = 0.047$ [low vs high] [Figure S11A]), as well as among AD PPRS group for comparisons of p-tau181: ($p = 0.00072$ [low vs high], $p = 0.027$ [medium vs high]; t-tau: $p = 0.0035$ [low vs high], $p = 0.039$ [medium vs high]; Aβ40: $p = 0.034$ [ANOVA]; Aβ42: $p = 0.022$ [low vs medium] [Figure S11B]). Similar but generally more significant associations of plasma AD biomarker expression were observed for comparisons in MCI and AD PPRS groups among CN participants (Figure S12).

3.6 | Enriched pathways and disease-specific proteins

GO biological process analysis was carried out on 36 and 50 proteins involved in MCI and AD-specific PPRS, respectively. Myeloid leukocyte migration is the most enriched pathway in MCI proteins (Figure 5A), while chemotaxis is the top significantly enriched pathway in AD proteins (Figure 5B). There were several pathways, including migration, ERK1 and ERK2 cascade, chemotaxis, and inflammation, enriched in both MCI and AD proteins. Among the five common proteins (KIT, CHIT1, HGFA, AGER, MMP12), AGER and KIT were downregulated in MCI/AD, and CHIT1, HGFA, MMP12 were upregulated in MCI/AD compared to CN (Figure S13). AGER, also known as RAGE, is reported to be associated with diabetes and AD, and its level in circulating immune cells is fundamental for hippocampal inflammation and cognitive decline.[54]

Further, we tested the 36 MCI and 50 AD proteins to find how many were truly disease-specific and not associated with age. After multiple

TABLE 2 Association of PPRSs with MCI and AD incidences using Cox regression model.

Outcome	Models	Covariate	β	HR (95% CI)	*P*	C-index (%)
MCI (n = 1387 and n_event = 129)	**Reference**	Age	0.12	1.13 (1.10, 1.15)	2.8e-25	78.80
		Sex (female)	−0.43	0.65 (0.46, 0.92)	0.02	
		Education	−0.05	0.95 (0.89, 1.02)	0.19	
		APOE ε4 (1)	0.31	1.36 (0.90, 2.06)	0.14	
	Model 1	**MCI PPRS**	**1.94**	**6.97 (5.34, 9.12)**	**6.7e-46**	**82.52**
	Model 2	**MCI PPRS**	**1.65**	**5.20 (3.82, 7.08)**	**1.5e-25**	**84.61**
		Age	0.06	1.07 (1.04, 1.09)	8e-08	
		Sex (female)	0.00	1.00 (0.70, 1.43)	0.99	
		Education	−0.01	0.99 (0.93, 1.07)	0.87	
	Model 3	**MCI PPRS**	**1.65**	**5.22 (3.83, 7.12)**	**1.4e-25**	**84.80**
		Age	0.07	1.07 (1.04, 1.10)	3.8e-08	
		Sex (female)	0.01	1.01 (0.70, 1.44)	0.97	
		Education	−0.01	0.99 (0.93, 1.07)	0.89	
		ApoE ε4 (1)	0.33	1.39 (0.92, 2.09)	0.12	
	Reference	Age	0.19	1.21 (1.18, 1.24)	**6.8e-42**	88.73
AD (n = 1386 and n_event = 128)		Sex (female)	0.39	1.48 (1.00, 2.18)	0.05	
		Education	−0.04	0.96 (0.89, 1.04)	0.35	
		APOE ε4 (1)	1.07	2.92 (2.01, 4.25)	1.9e-08	
	Model 1	**AD PPRS**	**1.75**	**5.74 (4.67, 7.05)**	**5.6e-62**	**88.15**
	Model 2	**AD PPRS**	**1.31**	**3.70 (2.83, 4.85)**	**2.1e-21**	**90.64**
		Age	0.11	1.11 (1.08, 1.15)	1.5e-11	
		Sex (female)	−0.37	0.69 (0.46, 1.05)	0.09	
		Education	−0.02	0.98 (0.91, 1.06)	0.64	
	Model 3	**AD PPRS**	**1.30**	**3.67 (2.79, 4.82)**	**1.7e-20**	**91.28**
		Age	0.12	1.12 (1.09, 1.16)	4.2e-13	
		Sex (female)	−0.33	0.72 (0.47, 1.09)	0.12	
		Education	−0.02	0.98 (0.90, 1.06)	0.55	
		APOE ε4 (1)	0.98	2.65 (1.83, 3.85)	3e-07	

Abbreviations: AD, Alzheimer's disease; beta, regression coefficient; CI, 95% confidence interval for HR; HR, hazard ratio; MCI, mild cognitive impairment; n, sample size; n_event, event number; PPRS, plasma protein risk score.

corrections, six MCI and 13 AD proteins turned out to be associated with MCI and AD incidences, respectively, and not associated with age, including HGFA, which is in both MCI and AD PPRSs (Table S5).

3.7 | Validation of MCI PPRS and AD PPRS in independent proteomics data sets

A subset of 13 proteins (seven from MCI PPRSs [RPSA, MDH1, IL1B, PSMA2, DKK3, ECM1, and C3], five from AD PPRS [SPON1, ATP5F1B, ADAMTS15, ICAM2, and CXCL11], and one from both [CHIT1]) found in the ADNI CSF proteomics data were statistically significant with AD incidence from MCI (Figure 6A). PPRS based on these proteins is significantly associated with AD incidence (HR = 1.63 [1.4, 1.9], p = 2.7e-9 and C-index = 71.82%) adjusting for age, sex, education, and APOE ε4 (Figure 6A). Kaplan–Meier plots indicate that high-PPRS individuals have a higher risk of developing AD (p = 6.8e-11), supporting our findings (Figure 6B). Further, AD risk factors, including CSF Aβ42 (p = 4.6e-6 [low vs high], p = 0.00024 [medium vs high]), MMSE (p = 0.00071 [low vs high], p = 0.00064 [medium vs high]), and hippocampus volume (p = 0.034 [low vs medium], p = 2.9e-7 [low vs high], p = 0.00071 [medium vs high]) were significantly decreasing and CSF p-tau181 (p = 0.0082 [low vs medium], p = 1.3e-7 [low vs high], p = 0.0075 [medium vs high]), CSF t-tau (p = 0.012 [low vs medium], p = 3.6e-7 [low vs high], p = 0.008 [medium vs high]), CDRSB (p = 0.045 [low vs high]), and AV45 (p = 5.8e-5 [low vs high], p = 0.0065 [medium vs high]) significantly increasing in high-PPRS individuals (Figure 6B). A subset of eight proteins (three from MCI PPRS [IL1B, CAPG, and RPSA], four

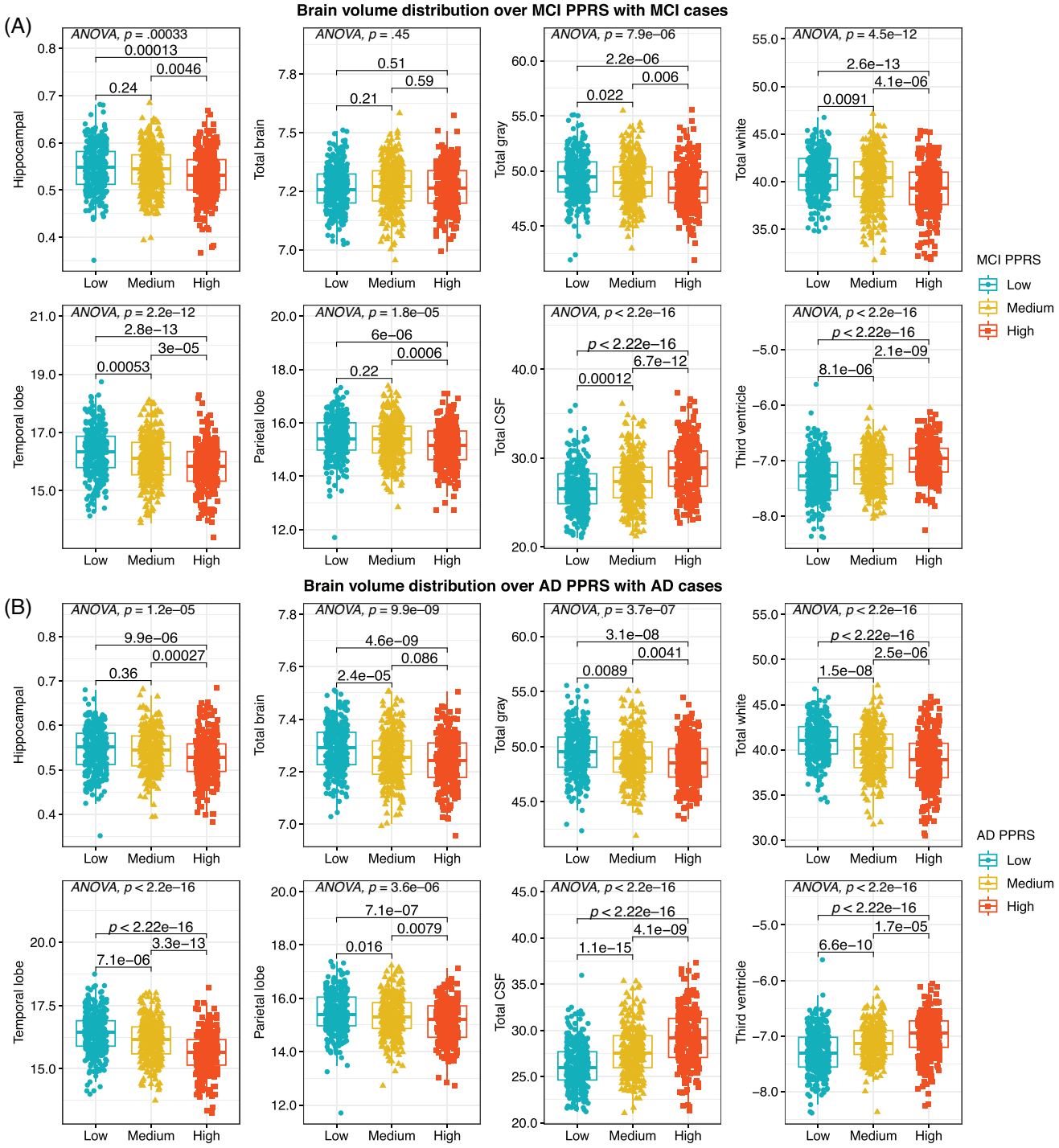

FIGURE 3 Brain volume distribution over (A) MCI PPRS and (B) AD PPRS with MCI and AD cases. MCI, mild cognitive impairment; AD, Alzheimer's disease; PPRS, plasma protein risk score; CSF, cerebrospinal fluid.

from AD PPRS [PRTN3, ANXA1, PDE5A, and CDK2], and one from both [MMP12]) was also statistically significant with MCI incidence from CN (Figure S14A). PPRS based on these proteins was significantly associated with MCI incidence (HR = 1.67 [1.33, 2.09], p = 1e-5) with a reasonable prediction power (C-index = 74.6%) after adjusting for age, sex, education, and APOEE ε4 (Figure S14A). Higher-PPRS individuals experiencing a higher risk of MCI (Kaplan–Meier curve, p = 2.6e-7) and having increasing levels of CSF p-tau181 (p = 0.0023 [low vs high]), CSF t-tau (p = 0.0039 [low vs high]), and AV45 (p = 0.0095 [low vs medium], p = 0.022 [low vs high]) (Figure S14B). In internal validation in FHS testing data set both MCI PPRS (HR = 2.28 [1.21, 4.31], p = 0.01) and AD PPRS (HR = 2.82 [1.78, 4.47], p = 9.4e-6) were statistically significant without adjusting for covariates and having reasonable C-indexes (MCI PPRS = 66.51% and AD PPRS = 78.65%) (Figure S15A). How-

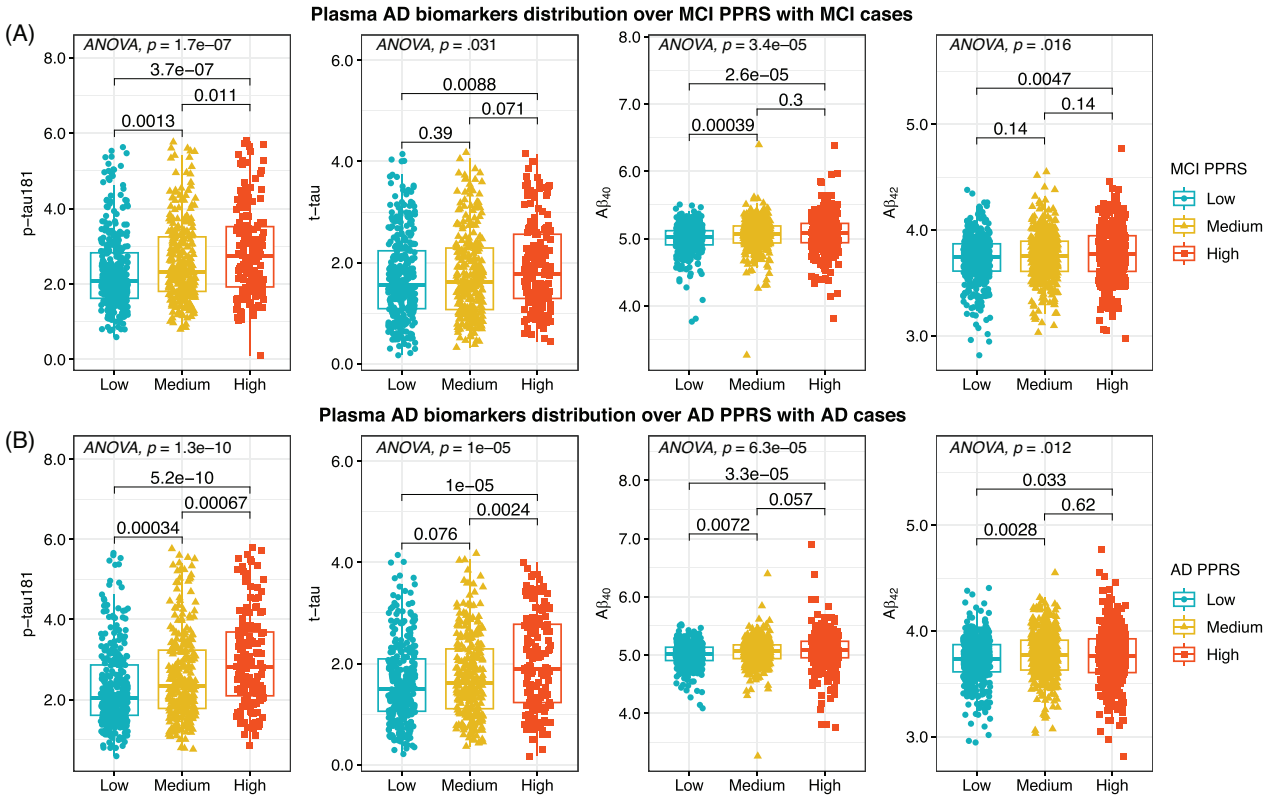

FIGURE 4 Distribution of plasma AD biomarkers over the different levels of (A) MCI PPRS and (B) AD PPRS. MCI, mild cognitive impairment; AD, Alzheimer's disease; PPRS, plasma protein risk score.

ever, these results were not significant after adjusting for age and sex (not included). But AD PPRS remains significant (HR = 2.14 [1.28, 3.59], $p = 0.0039$, and C-index = 80.34) after adjusting for FHS CVD risk score (Figure S15A). Kaplan–Meier curves also showed that high MCI PPRS ($p = 0.03$) and AD PPRS ($p = 3.2e\text{-}5$) individuals were at high risk of incidence of MCI and AD, respectively (Figure S15B).

4 | DISCUSSION

In this study, we developed the MCI and AD PPRSs to predict the risk of AD. Early diagnosis of AD is critical for initiating symptomatic therapy with antidementia medications. This will be even more important in the discovery of a biomarker that might predict MCI risk to aid in preventing and slowing AD progression. Established ATN AD biomarkers can differentiate AD from CN individuals and predict the likelihood of AD progression in MCI patients.[2,8,55] However, to our knowledge, no biomarkers can predict the incidence of MCI in CN individuals. This study provides evidence that from peripheral proteins, MCI PPRS predicted the incidence of MCI in CN individuals on average 18 years before onset with ideal predictive power (C-index = 82.52%) and slightly improved to 84.8% after adding age, sex, education, and APOE ε4 genotype (Table 2). Higher MCI PPRS is also useful for predicting cognitive changes, brain atrophy, especially in the hippocampus, and increasing levels of plasma AD biomarkers on an average of approximately 8 years and 12 to 19 years before onset, respectively (Figures 3 and 4). Since plasma biomarkers are inexpensive and have high predictive power for the preclinical stage of AD, they could be useful for clinical trials for novel drug discoveries. Although our AD PPRS results were better than the established risk score models that were developed for dementia outcomes in previous studies, for example, the dementia screening indicator [C-index = 68% (Cardiovascular Health Study), 77% (FHS), 76% (Health and Retirement Study), and 78% (Sacramento Area Latino Study on Aging)][56], a basic dementia risk model [C-index = 78%][57] and a clinical risk score for dementia reported a C-index of 85% for men and 87% for women.[58]

This study reveals that older age is associated with high MCI and AD PPRSs with respect to predicting MCI and AD incidences after 10, 15, and 20 years of follow-up (Figure S5). Individuals with low PPRSs in any age group are less likely to develop incidence of MCI and AD (Figure S5). MCI and AD PPRSs significantly negatively correlated with cognitive decline in three different domains, including memory, language, and executive function, as well as the global cognitive function MMSE score at four different subsequent exams (Figures S6 to S8). Both MCI and AD PPRSs equivalently predict memory decline, while AD PPRS has a strong association with language and executive function. Memory and language dysfunction are well-known defining characteristics of AD, and executive function is known to be linked with the frontal lobe. Our findings suggest that the tracking of PPRS would be able to

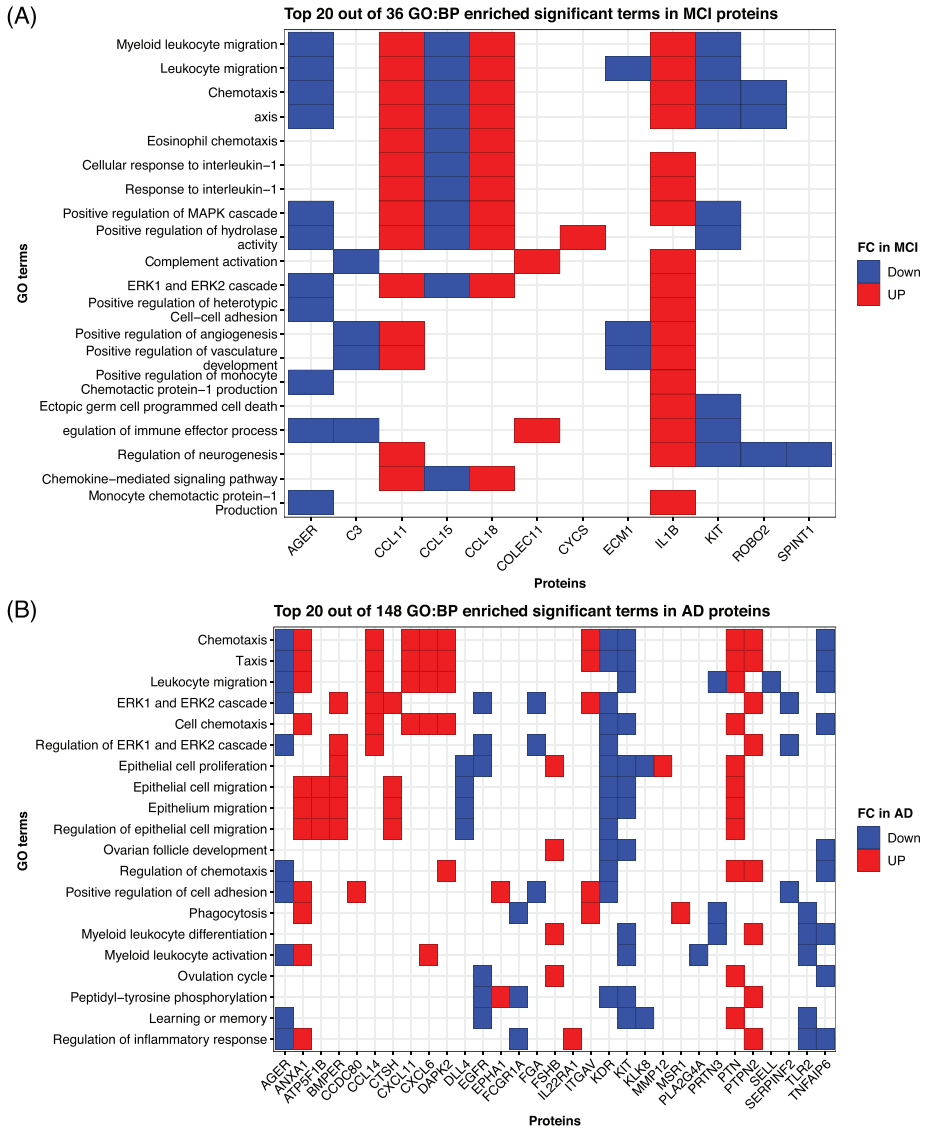

FIGURE 5 Heatmap plot for top 20 enriched significant GO terms (FDR < 0.05) in (A) MCI proteins and (B) AD proteins. The most significant terms are arranged from top to bottom on *y*-axis. ClusterProfiler returned several over-represented similar GO terms, but only the unique terms are shown here. FC denotes fold change in protein expression among MCI/AD versus CN. GO:BP Gene Ontology: Biological Process.

predict the early changes in cognitive decline and help delay or manage the onset of MCI and AD. Further, it is also noticed that sex is significantly associated with MCI incidence (reference model: $p = 0.02$) and marginally associated with AD incidence (reference model: $p = 0.05$). However, when MCI and AD PPRSs were included in models 1 to 3 (Table 2), the effect direction reversed; this may have been due to the fact that the MCI PPRS significantly decreased while AD PPRS increased in females (Figure S16). More investigations are required in this direction.

In high MCI and AD PPRSs, hippocampal volume, total gray matter, total white matter, temporal lobe, and parietal lobe were significantly decreased, while total CSF and third ventricle increased. Reduction in hippocampus volume is related to cognitive impairment and AD neuropathological markers, and the rate of hippocampal volume loss can be evaluated by MRI.[59,60] Total gray and white matter fluctuate at various stages of AD[61] and the gray-to-white matter signal ratio is a unique matrix of neurodegeneration in AD.[62] The temporal lobe is the epicenter of AD pathology, especially in classic late-onset cases.[63] It has been demonstrated that early in the course of MCI, when memory problems and hippocampal atrophy are less obvious, there may be hyperactivation of medial temporal lobe (MTL) circuits, which could indicate ineffective adaptive function.[64] Metabolic restrictions and physical developmental alterations in modern humans' medial parietal areas may play a role in early AD onset.[65,66] CSF volume increased linearly due to the aging effect.[67] In AD patients, the large third ventricle indicates an extent of cholinergic impairment rather than the severity of histological alterations, plaque scores, and tangles.[68] Our findings indicate that MCI and AD PPRSs might detect an early brain atrophy measured by MRI. As a result, PPRS can be used as an initial screening technique to assess patient AD risk. If a positive result

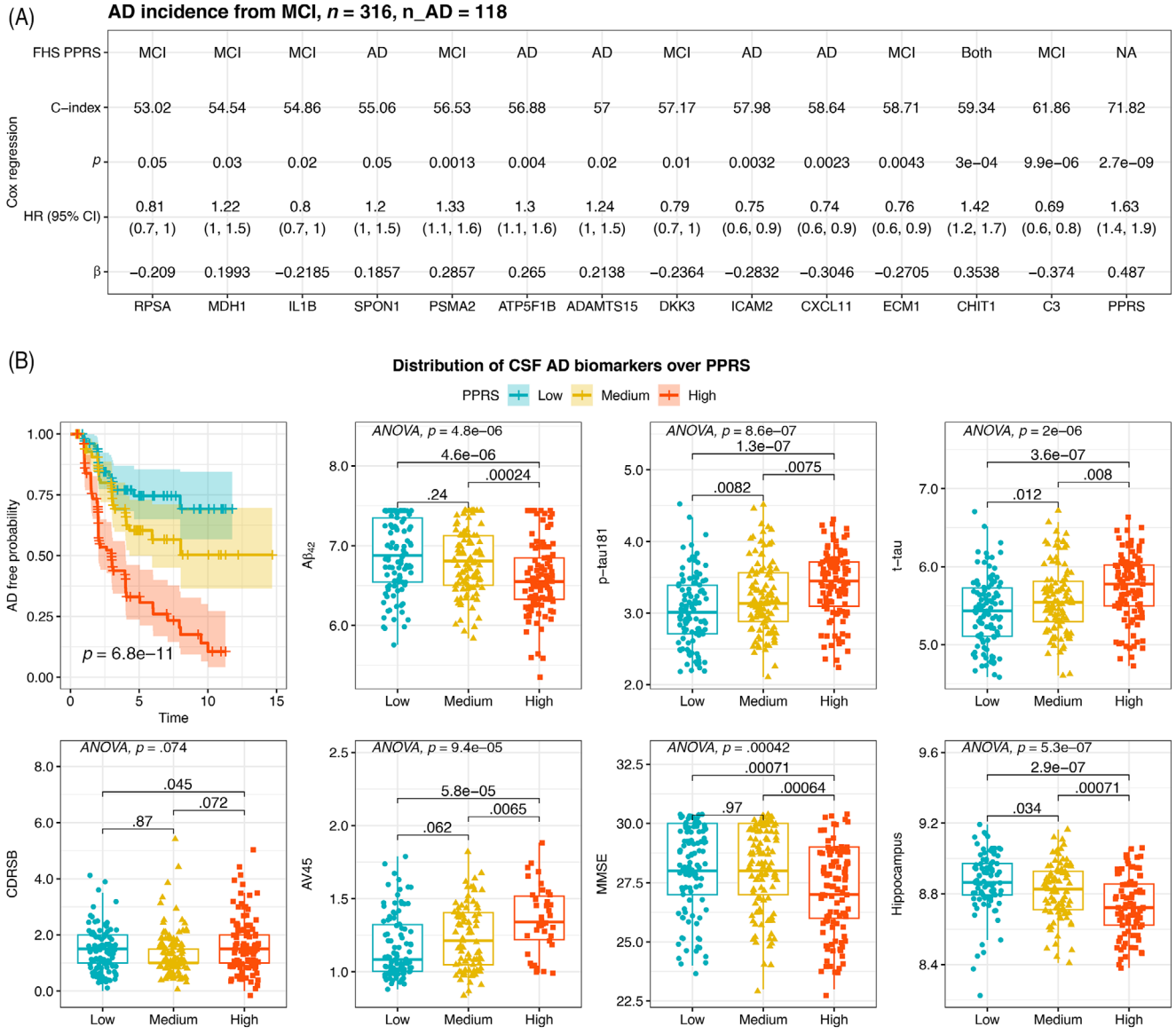

FIGURE 6 Validation in ADNI CSF proteomics data set. (A) Association of 13 proteins and aggregated protein risk score (PPRS) with incidence of AD from MCI. PPRS was calculated using sum of product of regression coefficients (β) with respective protein expression levels. The FHS PPRS row indicates that proteins belong to MCI or AD PPRS in the FHS training set. (B) Comparison of low-, medium-, and high-PPRS individuals based on CSF AD biomarkers and AD risk factors at baseline. PPRS in low, medium, and high were grouped based on tertiles. AV45 ratio of cortical gray matter and whole cerebellum. Summary florbetapir cortical SUVR normalized by whole cerebellum. CSF Aβ42, p-tau181, t-tau, and hippocampus volume were log transformed. MCI, mild cognitive impairment; AD, Alzheimer's disease; PPRS, plasma protein risk score; FHS, Framingham Heart Study; HR, hazard ratio; CDRSB, Clinical Dementia Rating scale Sum of Boxes; MMSE, Mini-Mental State Examination.

is obtained, patients will be advised to undergo additional expensive and invasive tests, such as CSF and PET scans, to confirm AD or related outcomes.

Interestingly and consistently, higher MCI and AD PPRSs resulted a linear increase in plasma AD biomarkers levels measured after roughly 8 to 19 years of protein measurement (Figure 4). Significant attention has been devoted to plasma-based biomarkers for AD diagnosis and AD-related outcomes, especially plasma p-tau217 and p-tau181.[4,12,27,28,69] Plasma p-tau217 alone in the BioFinder cohort has been shown to predict (area under the curve [AUC] = 83%) the progression of AD within 4 years in individuals with subjective cognitive impairment and MCI.[27] Despite the increased popularity of plasma-based p-tau biomarkers for AD outcomes, no consistent and approved model has been developed, and underlying molecular pathways are unclear. Several studies have uncovered various subsets of plasma proteins that can predict the risk of AD and accurately distinguish AD from normal cognition.[29–32,36] Also, our recent study determined that plasma-based proteins performed better than commonly measured CSF proteins and CSF AD biomarkers.[11] These studies had limited overlap between the lists of proteins, perhaps one or two. As a result, such studies required replication with a large sample size and longer follow-up times.

According to the National Institute on Aging and Alzheimer's Association (NIA-AA) 2018 research framework, AD is characterized biologically by neuropathological changes or biomarkers, and cognitive impairment is treated as a symptom of the disease rather than as a disease definition.[70] An interesting aspect of the 2018 NIA-AA research framework is the ability to incorporate or add more biomarkers to the ATN classification.[2] As a result, the ATN(X) classification is created, with X potentially representing a new biomarker category in addition to ATN. Inflammatory/immune processes (I), vascular brain damage (V), and alpha-synucleinopathy (S) are three potential novel biomarker categories that have yet to be confirmed. Because AD frequently coexists with other diseases in older persons, V and S biomarkers are important in its diagnosis and progression.[71] It would also be worthwhile to examine whether the combination of plasma p-tau217 or p-tau181 and the PPRS proteins further improved the sensitivity and specificity of MCI and AD. Our findings suggest that the efficacy of MCI and AD PPRSs in diagnosing clinical MCI and AD incidence and their partial validation in CSF in an independent cohort, give strong evidence for the protein risk score's therapeutic significance.

GO analysis showed that MCI and AD proteins shared several pathways, for example, "leukocyte migration" and "ERK1 and ERK2 cascade." Recent studies have shown that Aβ accumulation in the vascular system affects the expression of tight junction proteins and adhesion molecules in AD-like pathogenesis, potentially allowing circulating leukocytes to cross the barrier.[72,73] ERK1 and ERK2 are dysregulated in AD patients, potentially contributing to the disease's pathologies, such as Aβ plaque formation, tau phosphorylation, and neuroinflammation.[74] Most of the critical pathways enriched in MCI genes are also related to the immune systems. Adaptive immunity, useful in responding to injury and certain central nervous system disorders, may also contribute to neuroinflammation in AD.[75] IL1B, KDR, and KIT are the most frequently linked proteins in MCI and AD-enriched pathways, respectively. Multiple studies show that IL1B is a cytokine with a significant modulatory impact on AD pathogenesis[76], and higher levels of IL-1 expression have been linked to AD.[77] KDR, also referred to as vascular endothelial growth factor receptor 2 (VEGFR2), was initially discovered to be an essential regulator of angiogenesis and also known to mediate the migration, proliferation, permeability, and survival of endothelial cells.[78] KIT is a receptor tyrosine kinase that was initially developed to treat hemato-oncological diseases and is now being studied for the therapy of non-oncological diseases such as asthma, rheumatoid arthritis, and AD, among others.[79] Furthermore, we found that HGFA was a common protein involved in MCI and AD PPRSs that could serve as a disease-specific marker regardless of age (Table S5). Given this, our study and others suggest that the peripheral blood–brain axis plays an important role in AD development and progression.

Despite numerous promising results, our study has some limitations. Even though we found that PPRS could predict MCI and AD incidences and that higher PPRS may influence brain volume loss and increase plasma AD biomarkers level in FHS, we lacked an external independent cohort with plasma proteomics to validate. As a result, a replication study evaluating the behavior of MCI and AD PPRSs in plasma/blood from a large population is needed to confirm the findings. Furthermore, the FHS cohort is an ethnically homogeneous population of white individuals with European ancestry, and results are not generalizable to individuals from different ethnic and racial backgrounds. Since FHS does not have CSF data, we were unable to correlate PPRS performance with established CSF AD biomarkers, such as p-tau and Aβ42. Also, plasma AD biomarkers and SOMAscan proteomics profiling in FHS were not measured at the same time point, which limited the comparison of PPRS plasma AD biomarkers in predicting the risk of MCI and AD incidences, specifically, p-tau217 or p-tau181, which recently have been found to accurately predict the risk of AD, cognitive decline, and conversion to AD in a diverse population.[80]

In summary, our large-scale plasma proteomics study suggests that higher PPRS can be used as a risk predictor of MCI and AD incidences and related outcomes. Therefore, an aggregative protein risk score derived from a single plasma sample could be considered a cost-effective and scalable potential biomarker of MCI and AD and help individuals to prevent AD and slow its progression. In addition, we identified several pathways, for example, leukocyte, chemotaxis, migration, ERK1 and ERK2, and immune pathways that could be potential contributors to AD pathogenesis.

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ing of this report. A complete listing of ADNI investigators can be found at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf. This study was supported by the Framingham Heart Study's National Heart, Lung, and Blood Institute contract N01-HC-25195; NIA grants U19-AG068753, RF1AG075832-01A1, U01-AG072577, and R01-AG080810; Framingham Heart Study Brain Aging Program (FHS-BAP) pilot grant U19-AG068753; and the National Science Foundation grant DMS/NIGMS-2347698.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Rhoda Au is a scientific advisor to Signant Health and NovoNordisk. Author disclosures areavailable in the Supporting Information.

CONSENT STATEMENT

Informed consent was obtained from all study participants, and the Institutional Review Board of Boston University approved the study protocol.

DATA AVAILABILITY STATEMENT

The data set used in the preparation of this manuscript provided by the FHS-BAP and data is available on request. Please visit FHS-BAP website for more information https://www.bumc.bu.edu/fhs-bap/

ORCID

Habbiburr Rehman https://orcid.org/0000-0003-1762-3573

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SUPPORTING INFORMATION

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

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