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RESEARCH ARTICLE

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Plasma proteomics for cognitive decline and dementia—A Southeast Asian cohort study

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Abstract

INTRODUCTION: The prognostic utility of plasma proteomics for cognitive decline and dementia in a Southeast Asian population characterized by high cerebrovascular disease (CeVD) burden is underexplored.

METHODS: We examined this in a Singaporean memory clinic cohort of 528 subjects ($n = 300$, CeVD; $n = 167$, incident cognitive decline) followed-up for 4 years.

RESULTS: Of 1441 plasma proteins surveyed, a 12-protein signature significantly predicted cognitive decline (q-value $< .05$). Sixteen diverse biological processes were implicated in cognitive decline. Ten proteins independently predicted incident dementia (q-value $< .05$). A unified prediction model combining plasma proteins with clinical

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risk factors increased the area under the curve for outcome prediction from 0.62 to 0.85. External validation in the cerebrospinal fluid proteome of an independent Caucasian cohort replicated four of the significantly predictive plasma markers for cognitive decline namely: GFAP, NEFL, AREG, and PPY.

DISCUSSION: The prognostic proteins prioritized in our study provide robust signals in two different biological matrices, representing potential mechanistic targets for dementia and cognitive decline.

KEYWORDS
blood biomarker, cerebrovascular disease, cognitive decline, incident dementia, proteomics, Southeast Asian

Highlights

- A total of 1441 plasma proteins were profiled in a Singaporean memory clinic cohort.
- We report prognostic plasma protein signatures for cognitive decline and dementia.
- External validation was performed in the cerebrospinal fluid proteome of a Caucasian cohort.
- A concordant proteomic signature was identified across both biofluids and cohorts.
- Further studies are needed to explore the therapeutic implications of these proteins for dementia.

1 | BACKGROUND

Dementia is a devastating consequence of aging, associated with high health care expenditure and decline in health-span.[1] Blood-based biomarkers of cognitive decline afford the opportunity to screen at-risk patients, administer early targeted treatment to modify disease trajectory, and identify novel therapeutic targets.

Current biomarkers of dementia pathophysiology, particularly amyloid and tau proteins, which characterize Alzheimer's disease (AD), have been researched extensively, and targeting these proteins in therapeutic trials has been met with limited success.[2–4] However, longitudinal studies suggest that the majority of individuals who are amyloid positive but cognitively normal may well avoid dementia in their lifetime.[5] Indeed, it has been increasingly recognized that apart from AD, diverse pathophysiological processes involving inflammation, extracellular matrix disruption, and vascular dysfunction can also contribute to age-related neurocognitive decline.[6–8]

Advances in plasma proteomics allow for large-scale biomarker screening to identify diverse proteins (beyond amyloid and tau) underpinning cognitive disease phenotypes. However, current studies exploring plasma proteomics with respect to longitudinal cognitive outcomes, conducted predominantly in Caucasian populations, are focused largely on their role as mid-life indicators of disease.[9–16] It remains unclear whether these protein biomarkers of cognitive decline in younger populations remain applicable to a higher-risk Asian patient group burdened with cerebrovascular disease (CeVD) and concomitant neuropathology.[17] Asian populations, characterized by an increased CeVD burden, have been underrepresented in earlier studies, and conceivably, ethnic factors may affect the prognostic utility of conventional biomarkers amyloid and tau, or neurodegeneration.[18,19] Therefore, there remains a need to investigate plasma protein biomarker signatures of cognitive decline and dementia in Asian populations with an elevated prevalence of concomitant CeVD.

To this end, we applied plasma proteomic profiling to identify novel circulating proteins predictive of cognitive decline and progression to dementia in a well-annotated Southeast Asian memory clinic cohort. We hypothesized that diverse proteomic biomarkers reflect the multi-faceted pathophysiology of cognitive decline and dementia, including neurodegeneration, inflammation, vascular dysfunction, and extracellular matrix remodeling. To investigate the pathophysiological relevance of these prognostic proteins, we evaluated their associations with neuroimaging markers of neurodegeneration and cerebral small vessel disease (CSVD). Subsequently, we validated select components of the high-throughput proteomic platform with use of quantitative immunoassays. Finally, we stringently filtered the top protein candidates by benchmarking plasma proteins for cognitive decline against the cerebrospinal fluid (CSF) proteome of an independent external validation cohort.

2 | METHODS

2.1 | Primary study cohort

The primary study cohort (Memory Aging and Cognition Centre [MACC] Harmonisation Cohort) comprised subjects recruited from

memory clinics from two Singaporean study sites (National University Hospital and St Luke's Hospital) and followed prospectively for up to 4 years. Subjects were ≥50 years of age, with sufficient language skills to participate in neuropsychological assessments. Exclusion criteria were major psychiatric illness, substance abuse disorder, traumatic brain injury resulting in cognitive impairment, tumors, multiple sclerosis, and epilepsy. The detailed study protocol has been described previously.[20] Informed written consent was obtained prior to study recruitment. Ethics approval for this study was obtained from all participating institutions, including the National Healthcare Group Domain Specific Review Board (NHG DSRB reference numbers 2018/01098 and 2010/00017). The study design is outlined in Figure 1.

2.2 | Assessment of clinical demographics

Data from all participants, including age, gender, education level, lipid-lowering medications, antihypertensive medications, and clinical risk factors including hypertension, hyperlipidemia, obesity (defined as a body mass index [BMI] of ≥30 kg/m^2), and diabetes, were collected via detailed questionnaires administered in a standardized fashion.[21] Apolipoprotein E (*APOE*) ε4 status was determined using genotyping, as described previously.[22] Subjects with at least one *APOE* ε4 allele were defined as *APOE* ε4 carriers.

2.2.1 | Neurocognitive assessments

Neurocognitive diagnoses were ascertained at regular consensus meetings attended by study clinicians and neuropsychologists, during which clinical neurocognitive and brain neuroimaging data were reviewed. Yearly cognitive assessments, using a locally validated neuropsychological test battery, were administered in the subject's native language (English, Mandarin, or Malay) by trained research psychologists, as described previously.[23] Subjects without objective cognitive impairment were categorized as no cognitive impairment (NCI). Subjects who displayed impairment in one or more cognitive domains (defined by a score of at least 1.5 standard deviations [SDs] below established education-adjusted cutoff values on any test) but did not exhibit loss of independent daily function were classified as cognitive impairment no dementia (CIND).[20] Dementia was diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Supplementary Methods 1 provides details on cognitive testing. To stage the severity of cognitive impairment and dementia, Clinical Dementia Rating (CDR) scores were assigned at baseline and at every follow-up visit.[24]

The primary outcome was incident cognitive decline, defined by a longitudinal change in CDR Sum of Boxes (CDR-SB) score of ≥3 points at any annual follow-up, compared to the baseline. The CDR-SB was employed as a clinically important indicator of cognitive impairment due to its validation as a marker of longitudinal cognition and function, whereas the cutoff score accords with minimal clinically important differences.[24,25]

RESEARCH IN CONTEXT

1. **Systematic review**: Advances in plasma proteomics allow for large-scale screening for the identification of diverse biomarkers of cognitive decline. However, the utility of plasma proteomics for the prediction of cognitive decline and incident dementia in an at-risk Southeast Asian population characterized by high cerebrovascular disease (CeVD) burden remains underexplored.
2. **Interpretation**: We profiled 1441 plasma proteins, identifying prognostic proteomic signatures underpinning cognitive decline and incident dementia in a Singaporean memory clinic cohort with high CeVD burden. These plasma proteins added significant incremental predictive value to clinical factors for the prediction of future cognitive outcomes.
   External validation in the cerebrospinal fluid proteome of an independent longitudinal Caucasian cohort replicated common prognostic proteins for cognitive decline, concordantly altered across both biofluids.
3. **Future directions**: Mechanistic studies of these prognostic proteins should be undertaken to elucidate their utility as potential therapeutic targets in the management of cognitive decline and incident dementia.

As a secondary outcome, we evaluated the progression from CIND to incident dementia defined by a change in CDR Global Score (CDR-GS) from 0 or 0.5 to ≥1, across any time point of follow-up.[24] We decided to focus our analysis of incident dementia on subjects with CIND, in view of the lower risk of dementia in NCI subjects.

2.3 | Neuroimaging assessments

Baseline brain magnetic resonance imaging (MRI) scans were performed within 12 months of blood draw as described previously (refer to Supplementary Methods 2 for neuroimaging protocol).[20] The presence of CSVD markers, including lacunes, white matter hyperintensities (WMHs), cerebral microbleeds, cortical infarcts, and CeVD at baseline, was determined by an expert clinical rater (S.H.) as reported previously (Supplementary Methods 2).[20] Significant CeVD was defined as the presence of cortical infarcts, and/or two or more lacunes, and/or confluent WMHs in two regions of the brain (defined by the age-related white matter changes score ≥8) as reported previously.[20] Automated MRI brain volumetric analysis was performed for all subjects using AccuBrain (IV2.0) to obtain serial quantitative MRI volumetric markers of neurodegeneration (regional brain atrophy) and CSVD (WMH volume).[26] These were adjusted for total intracranial volume for analysis. The AccuBrain platform has been validated previously, demonstrating comparable accuracy with commonly used volumetric analytical platforms such as FreeSurfer.[26]

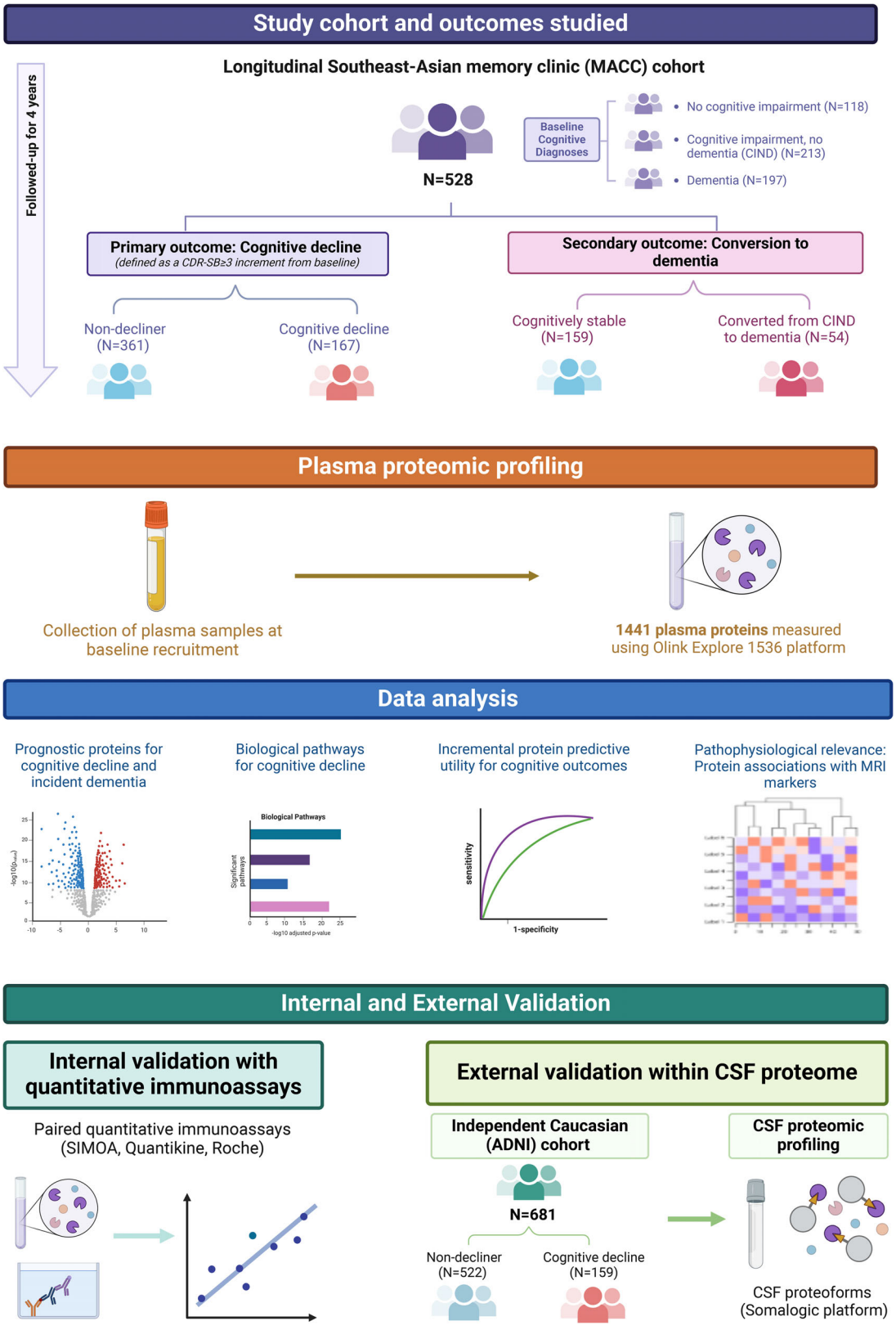

FIGURE 1 Overview of the study. CIND, cognitive impairment no dementia; NCI, no cognitive impairment; CDR-SB, Clinical Dementia Rating Sum of Boxes; CSF, cerebrospinal fluid. Created with biorender.com.

2.4 | Plasma proteomic profiling

Baseline levels of plasma proteins representing major biological pathways were analyzed using the Olink Explore 1536 platform (Thermo Fisher Scientific, Waltham, MA, USA). A total of 1441 proteins were used for statistical analysis after rigorous quality control of the data. Protein levels were expressed as Normalized Protein eXpression (NPX) values, a relative quantification unit logarithmically related to protein concentration. Details on the experimental protocol and proteins studied are detailed in the Supplementary Methods 3 and Table S1, respectively.

2.5 | Internal validation of Olink data with quantitative immunoassays

Internal validation of Olink biomarkers was performed using three quantitative immunoassay platforms for GDF-15 (Quantikine, R&D Systems, Inc, Minneapolis), NTproBNP (Roche Diagnostics GmbH), and NEFL (Single molecule array (SIMOA), Quanterix, Billerica, MA, USA; refer to Supplementary Methods 4 for experimental details).

2.6 | External validation in the CSF proteome of the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort

Plasma proteomic findings from the primary MACC study cohort were validated externally in an independent cohort (Alzheimer's Disease Neuroimaging Initiative [ADNI]) with publicly accessible longitudinal cognitive data and CSF proteomic data (from adni.loni.usc.edu). The ADNI database was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. In brief, the primary goal of ADNI was to test if serial MRI, positron emission tomography (PET), biological, and clinical or neuropsychological biomarkers can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. (Refer to Supplementary Methods 5 and 6 and www.adni-info.org for more details.) CSF proteomic profiling of the ADNI cohort was performed previously using the Somalogic 7K platform (refer to http://adni.loni.usc.edu/study-design/ for more details). A total of 681 eligible ADNI subjects with longitudinal cognitive follow-up and baseline CSF proteomic data were included for analysis. In accordance with the MACC cohort, the primary outcome of interest was cognitive decline, similarly defined as a CDR-SB 3-point increment from baseline at any longitudinal follow-up visit up to 4 years.

2.7 | Statistical analysis

Statistical analysis was performed using R version 4.3.2 (R Core Team. 2023. R Foundation for Statistical Computing, Vienna, Austria) and STATA (StataCorp. 2023. Release 18. College Station, TX: Statacorp LLC). All statistical tests were conducted at a significance level of .05. To account for false discovery rate, correction for multiple testing was performed with a q-value threshold of $< .05$ considered to be statistically significant, unless otherwise stated.[27]

2.8 | Cohort demographic comparisons

Patient characteristics were expressed as percentages for categorical variables and mean ± SD for continuous variables; chi-square tests were used in the analysis of categorical data. Independent t-tests were used in the analysis of normally distributed continuous variables. Demographic data for all patients were complete, except for two subjects without data on hypertension and hyperlipidemia. For these two subjects, the missing demographic data were imputed using the k-nearest neighbor method utilizing logistic regression imputation.

2.9 | Identification of predictive proteomic signatures for incident cognitive decline

The association of circulating proteins with the primary outcome of incident cognitive decline (as measured by a longitudinal increment of CDR-SB score of ≥3 points) was evaluated in both univariate (unadjusted) and multivariable Cox proportional hazards regression models, with the latter adjusted for clinically relevant risk factors including age, gender, hypertension, hyperlipidemia, diabetes, years of education, lipid-lowering medications, antihypertensive medication, obesity, *APOE* ε4 status, and baseline cognition expressed as CDR-GS. Time to event (event refers to cognitive decline) was specified as the point at which a participant's CDR-SB score had increased by ≥3 points. Proteins associated with the risk of cognitive decline were visualized as volcano plots. The prognostic utility of individual plasma proteins for cognitive decline was additionally evaluated using the Harrell's C-index. Over-representation analysis for the proteins associated with cognitive decline (p-value $< .05$, q-value $< .2$) was performed using the DAVID bioinformatics resource, with biological pathways mapped to Reactome and Gene Ontology terms.[28] Wherever applicable, multiple testing-adjusted p-values were presented. In addition, exploratory subgroup analysis was performed to examine the predictive performance of these proteins in the pre-dementia (comprising NCI and CIND subjects) and the dementia groups. Proteins significantly associated with the risk of incident cognitive decline within the subgroups were reported.

For multivariate modeling, regularized regression with an elastic net penalty was used to identify a model to predict cognitive decline, incorporating clinical characteristics and plasma proteins, using the '*glmnet*' library of R.[29] Predictive models of cognitive decline were constructed progressively using variables from pre-specified variable categories. Model 1: Clinical model comprising 10 clinical risk factors: age, gender, education, diabetes, hypertension, hyperlipidemia, *APOE* ε4 status, antihypertensive medication, lipid-lowering medication, and obesity;

Model 2: Clinical+cognition model expanding Model 1 by incorporating baseline cognitive performance expressed as CDR-GS; and Model 3: Clinical+cognition+protein model expanding Model 2 with all predictive plasma proteins for cognitive decline (p-value < .05, q-value < .2). Using the features identified in the elastic net regression model, nested cross-validation was used to evaluate the receiver-operating characteristic (ROC) curve expressed as AUROC (area under the ROC curve)) with 95% confidence intervals (CIs). The range of cross-validated error was compared using nested cross-validation (R package '*nestedcv*').[30] To determine the importance of features for the prediction of cognitive decline, Shapley additive values (SHAP) of the selected predictors were computed, and presented as SHAP bar plots using the '*fastshap*' R package.[31] To determine the predictive power of individual proteins, the AUROC of each protein was evaluated. Differences between the predictive performance in AUROCs were compared across the three progressively expanded models (using the *'roc.test'* function of R). The AUROCs were additionally reported within the pre-dementia and dementia cognitive subgroups.

2.10 | Identification of predictive plasma proteins for progression from CIND to incident dementia

The secondary cognitive outcome studied was the progression from CIND to incident dementia. Cox proportional hazards regression was used to evaluate associations of all Olink proteins with conversion to dementia, adjusted for clinically important covariates including age, gender, years of education, hypertension, hyperlipidemia, *APOE* ε4 status, antihypertensive medication, lipid-lowering medication, obesity, and diabetes. Time to event (event refers to progression to dementia) was specified as the point at which a participant with baseline CIND displayed an increase in CDR-GS score from 0 or 0.5 to ≥1. The combined prognostic utility of all significant plasma proteins, as compared with clinical factors for predicting incident dementia, was evaluated using Harrell's C-index.

2.11 | Secondary analyses

To complement the primary analyses, we conducted secondary analyses to test the associations between plasma proteins and the linear decline in cognitive function over time, as measured by CDR-SB scores. This analysis was conducted with stratification of subjects by baseline cognitive diagnosis (NCI, CIND, or dementia). A linear mixed-effects model with random intercepts was applied to leverage on the availability of yearly repeated cognitive assessments.[32] The model was adjusted for age, gender, education, hypertension, hyperlipidemia, diabetes, antihypertensive medication, lipid-lowering medications, *APOE* ε4 status, baseline cognition (CDR-GS), and the interaction of time x protein level. Plasma proteins significantly associated with steeper decline in cognitive function over time (q-value < .05 for the time x protein interaction term) were reported within each cognitive subgroup. To explore the biological pathways associated with the slope of decline in cognitive function over time in each cognitive subgroup, overrepresentation analyses of these significant proteins were performed and mapped to biological pathways using the DAVID bioinformatics resource. Multiple testing-adjusted p-values were reported.[28]

2.12 | Internal validation with quantitative immunoassays

Internal validation of assay performance was performed using quantitative immunoassays for GDF-15, NTproBNP, and NEFL, which were log-10 transformed prior to analysis to approximate a normal distribution. Pairwise correlations of Olink NPX values and their corresponding levels measured by immunoassay were presented as scatterplots. Correlation coefficients and p-values were calculated.

2.13 | External validation within the CSF proteome of the ADNI cohort

Analysis of patient characteristics and cognitive outcomes in the ADNI cohort followed a similar approach. Cox proportional hazards regression was employed to study associations of the candidate proteins within the CSF proteome with incident cognitive decline, adjusting for age, gender, cardiovascular diseases, baseline cognitive status, and years of education. Replicated markers of cognitive decline in the CSF proteome of the ADNI cohort and the plasma proteome of the MACC cohort were reported.

2.14 | Evaluation of associations of the predictive plasma proteins for cognitive decline with neuroimaging markers

The cross-sectional associations of the predictive plasma proteins with baseline MRI brain volumetric markers were studied in the MACC cohort. For CVSD markers, zero-inflated negative binomial regression was used in the analysis of count data (lacunes, cortical infarcts, cerebral microbleeds), whereas linear regression was used in the analysis of continuous variables (WMH volume). Models were adjusted for age, gender, education, hypertension, hyperlipidemia, and diabetes. The association structure was visualized using heatmaps of standardized regression coefficients, presented alongside adjusted p-values adjusting for false discovery rate.

3 | RESULTS

3.1 | Clinical characteristics

An overview of the study is presented in Figure 1. Among 528 subjects from the MACC cohort included for analysis (mean age 72.8 ± 7.8 years, 43.8% male), 57.9% had significant CeVD burden, 71.4% hypertension, 33.7% diabetes, 22.3% NCI, 40.3% CIND, and 37.3% dementia

TABLE 1 Distribution of demographic factors and clinical information in the MACC cohort.

Demographics (*n* (%) / mean ± SD)	All (*N* = 528)	Developed cognitive decline (*n* = 167, 31.6%)	Remained cognitively stable (*n* = 361, 68.4%)	*p*-value
Age, years	72.83 ± 7.81	74.71 ± 7.04	71.96 ± 8.00	<0.001
Education, years	7.06 ± 4.96	5.86 ± 4.48	7.62 ± 5.07	<0.001
CDR-GS	0.69 ± 0.70	1.05 ± 0.59	0.52 ± 0.68	<0.001
Gender (male)	231 (43.8)	63 (37.7)	168 (46.5)	0.058
Ethnicity				
Chinese	444 (84.1)	143 (85.6)	301 (83.4)	0.152
Malay	49 (9.3)	14 (8.4)	35 (9.7)	
Indian	29 (5.5)	6 (3.6)	23 (6.4)	
Others	6 (1.1)	4 (2.4)	2 (0.6)	
Baseline cognitive diagnosis				
NCI	118 (22.3)	6 (3.6)	112 (31.0)	<0.001
CIND	213 (40.3)	51 (30.5)	162 (44.9)	
Dementia	197 (37.3)	110 (65.9)	87 (24.1)	
Baseline CeVD	300 (57.9)	107 (66.9)	193 (53.9)	0.006
Hypertension	377 (71.4)	124 (74.3)	153 (70.1)	0.324
Hyperlipidemia	398 (75.4)	122 (73.1)	276 (76.5)	0.399
Diabetes	178 (33.7)	68 (40.7)	110 (30.5)	0.021
Antihypertensive medication use	342 (64.8)	105 (62.9)	237 (65.7)	0.535
Lipid-lowering medication use	271 (51.3)	88 (52.7)	183 (50.7)	0.669
Obesity	39 (7.5)	7 (4.3)	32 (9.1)	0.055
APOE ε4 status	156 (29.5)	55 (32.9)	101 (28.0)	0.246

Note: Obesity: defined as a body mass index of ≥30 kg/m^2.
Abbreviations: *APOE* ε4, apolipoprotein E ε4 allele; CDR, Clinical Dementia Rating; CeVD, cerebrovascular disease; CIND: cognitive impairment no dementia; MACC: Memory Aging and Cognition Centre; NCI: no cognitive impairment; SD, standard deviation.

at baseline (refer to Figure S1 for the flowchart of subject recruitment). Cohort demographics are presented in Table 1.

3.2 | Prognostic proteins and biological pathways for cognitive decline and conversion to dementia

3.2.1 | Protein signatures and pathways for incident cognitive decline

The incidence rate of cognitive decline was 31.6% (167/528) over a mean follow-up duration of 39.6 ± 12.0 months. Of the 1441 proteins studied, 12 proteins were significantly associated with the risk of incident cognitive decline, independent of baseline cognitive function (*q*-value < .05, Table 2, Figure 2A). NEFL and PPY were the two most predictive proteins for cognitive decline. Adjusted and unadjusted analyses for all 117 predictive proteins (*p*-value < 0.05, *q*-value < .2) are presented in Table S2. Overrepresentation analysis of these proteins implicated 16 biological pathways underpinning cognitive decline, including extracellular matrix organization, immune system dysregulation, and the regulation of insulin growth factor (IGF) transport and uptake by IGF binding proteins (IGFBPs) (Figure 2B).

Subgroup analysis stratified by baseline cognitive subgroup (pre-dementia vs dementia) was conducted using all predictive proteins (*p*-value < .05, *q*-value < .2). Proteins significantly associated with the risk of cognitive decline in the pre-dementia and dementia subgroups are presented in Tables S3 and S4. Of the 117 proteins evaluated, 37 proteins were significantly associated with cognitive decline in the dementia subgroup, whereas 28 proteins were significant within the pre-dementia subgroup. The two most significant proteins for cognitive decline in the dementia subgroup were lithostathine-1-beta (REG1B) and amphoterin-induced protein 2 (AMIGO2); and amphiregulin (AREG) and tumor-associated calcium signal transducer 2 (TACSTD2) in pre-dementia subjects. In addition, a five-protein signature was identified, which associated with the risk of cognitive decline in both subgroups (pancreatic prohormone [PPY]), interleukin-15 (IL15), granulocyte-macrophage colony-stimulating factor receptor subunit alpha (CSF2RA), glial fibrillary acidic protein (GFAP), and cadherin-related family member 5 (CDHR5); Figure S2).

Elastic net regression analysis yielded an optimal multivariable model comprising baseline cognition and 26 plasma proteins, achieving a cross-validated AUROC of 0.85 (95% CI: 0.82–0.88). This model significantly outperformed both the model based on clinical risk factors alone (AUROC 0.62, 95% CI: 0.57-0.67, *p*-difference < .0001;

TABLE 2 Twelve plasma proteins significantly associated with the risk of incident cognitive decline.

Assay	Description	aHR[a]	95% C.I., LL	95% C.I., UL	*p*-value	*q*-value	c-index
NCS1	Neuronal calcium sensor 1	3.18	1.99	5.10	1.41E-06	8.97E-04	0.773
GFAP	Glial fibrillary acidic protein	1.81	1.42	2.31	1.60E-06	8.97E-04	0.772
NEFL	Neurofilament light polypeptide	1.49	1.25	1.78	6.82E-06	2.55E-03	0.784
GUCA2A	Guanylin	1.84	1.40	2.42	1.10E-05	2.55E-03	0.773
TIMP4	Metalloproteinase inhibitor 4	1.85	1.40	2.43	1.14E-05	2.55E-03	0.773
PPY	Pancreatic prohormone	1.31	1.16	1.49	2.85E-05	5.32E-03	0.779
KLK4	Kallikrein-4	1.36	1.17	1.59	6.05E-05	9.69E-03	0.774
FAM3B	Protein FAM3B	2.10	1.40	3.15	3.12E-04	3.55E-02	0.772
HSPB6	Heat shock protein beta-6	1.67	1.26	2.21	3.33E-04	3.55E-02	0.773
TACSTD2	Tumor-associated calcium signal transducer 2	2.43	1.49	3.97	3.58E-04	3.55E-02	0.767
KRT19	Keratin, type I cytoskeletal 19	1.42	1.17	1.72	3.80E-04	3.55E-02	0.775
AREG	Amphiregulin	1.58	1.23	24	3.80E-04	3.55E-02	0.769

Abbreviations: aHR, adjusted hazard ratio; *APOE* ε4, apolipoprotein E ε4 allele; CDR, Clinical Dementia Rating; CDR-GS, CDR Global Score; LL, lower limit; UL, upper limit.

[a]aHRs and 95% confidence intervals (C.I.s) were derived from Cox proportional hazards regression adjusted for age, gender, hypertension, hyperlipidemia, diabetes, years of education, antihypertensive medication, lipid-lowering medication, obesity, *APOE* ε4 status, and baseline cognition expressed as CDR-GS. Predictive power of individual proteins was assessed using Harrel's C-index. Type 1 error rates with multiple testing correction were estimated using *q*-values.

Figure 3A), and the model constructed from clinical risk factors with baseline cognition (AUROC 0.77, 95% CI: 0.73–0.81, *p*-difference < .0001; Figure 3A). When we stratified by baseline cognitive subgroup, the AUROC for the predictive model was significantly higher in the pre-dementia group (AUROC 0.87, 95% CI: 0.82–0.91) compared to the dementia subgroup (AUROC 0.66, 95% CI: 0.58–0.74, *p*-difference < .0001; Figure S3). Of all constituent predictors, GFAP, PPY, and baseline CDR-GS exhibited the highest feature importance (Figure 3B). The coefficients in the elastic net regression models are provided in Tables S5–S7. Individual AUROCs of the 26 selected proteins are presented in Figure S4 and ranged from 0.53–0.72. The top seven proteins of the model (GFAP, PPY, KRT19, NEFL, metalloproteinase inhibitor 4 [TIMP4], inter-alpha-trypsin inhibitor heavy chain H3 [ITIH3] and neuronal calcium sensor 1 [NCS1]) already yielded a combined AUROC of 0.78 for cognitive decline.

3.2.2 | Predictive plasma proteins for incident progression from CIND to dementia

In the subset of subjects with CIND at baseline ($N = 213$), the incidence of progression to dementia was 25.4% ($N = 54$) over 41.5 ± 10.9 months of follow-up. Subject characteristics stratified by dementia progression status are presented in Table S8. After adjustment for age, gender, hypertension, hyperlipidemia, *APOE* ε4 status, obesity, antihypertensive medications, lipid-lowering medications, and diabetes, 10 proteins remained independently predictive for progression to dementia: AREG, NEFL, TACSTD2, midkine (MDK), GFAP, KLK4, mucin-13 (MUC13), R-spondin-1 (RSPO1), polymeric immunoglobulin receptor (PIGR), and secretogranin-1 (CHGB) (*q*-value < .05; Table 3, Figure 2C, unadjusted analysis in Table S9). Of these, AREG was the most significantly predictive protein for progression to dementia. The combined predictive model based on clinical factors (age, gender, education, hypertension, hyperlipidemia, diabetes, obesity, lipid-lowering medication, antihypertensive use, and *APOE* ε4 status) and all 10 significant proteins yielded a combined C-index of 0.82, a significant improvement from the model comprising only clinical factors (C-index 0.69), for the prediction of incident progression to dementia.

3.2.3 | Secondary analyses

Secondary analyses were performed to identify the proteins, which significantly associated with linear changes in cognitive function over time, as measured by CDR-SB scores, stratified by baseline cognitive diagnosis (NCI, CIND, or dementia). Plasma proteins associated with a steeper decline in cognitive function over time, across the NCI, CIND, and dementia subgroups, were identified using linear mixed-effects models and visualized in a Venn diagram (Figure S5A; all significant proteins for each subgroup presented in Tables S10–S12). Nineteen overlapping proteins were common across all three cognitive subgroups: isopentenyl-diphosphate delta-isomerase 2 (IDI2), glycosyltransferase 8 domain-containing protein 2 (GLT8D2), NEFL, group 10 secretory phospholipase A2 (PLA2G10), GUCA2A, calbindin (CALB1), ras GTPase-activating-like protein (IQGAP2), polypeptide N-acetylgalactosaminyltransferase 10 (GALNT10), NCS1, Kallikrien-1 (KLK1), ADH4 (all-trans-retinal dehydrogenase [NAD(+)] ADH4), aminoacylase 1 (ACY1), aggrecan core protein (ACAN), coiled-coil domain-containing protein 80 (CCDC80), cathepsin B

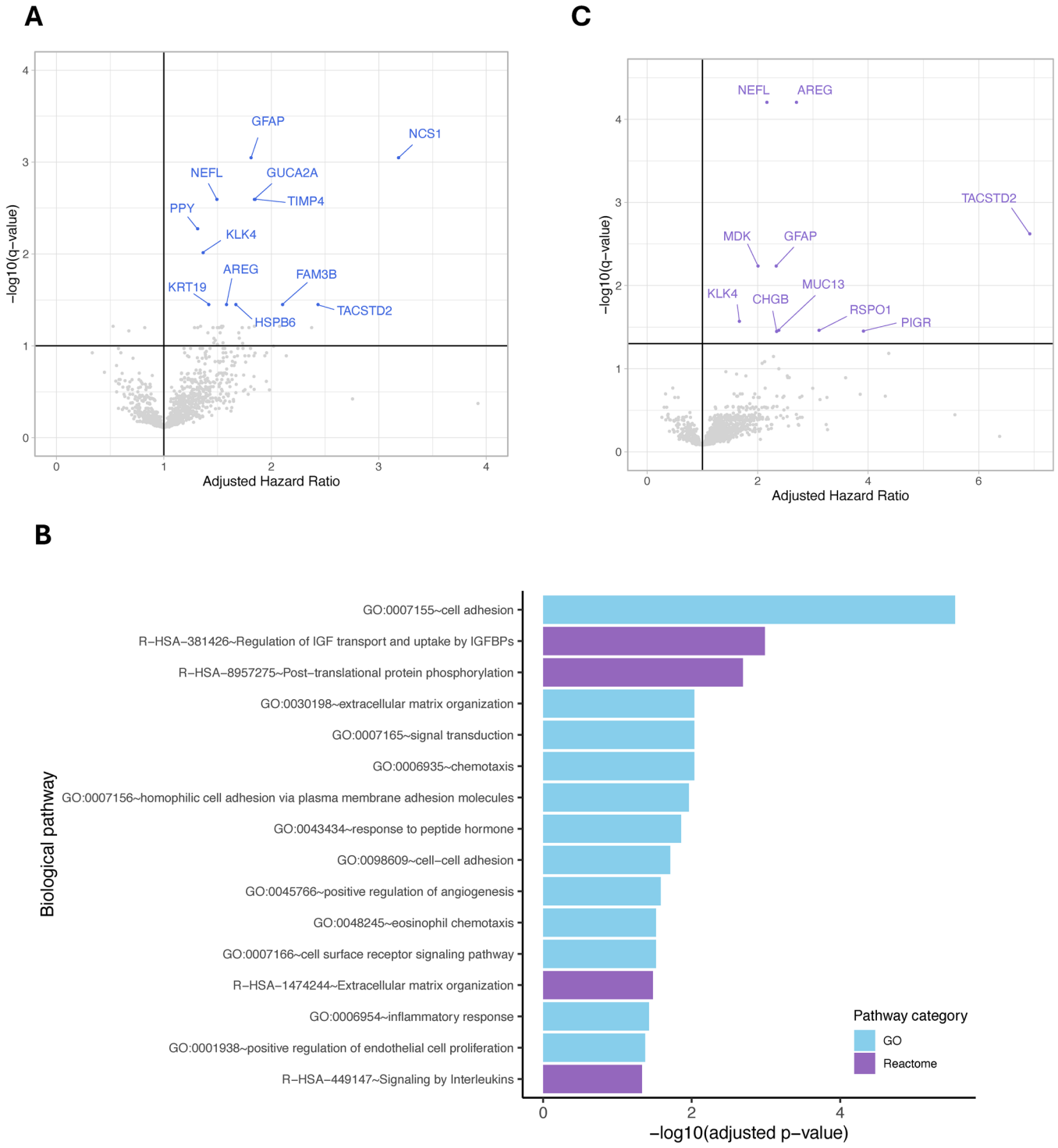

FIGURE 2 (A) Volcano plot of proteins significantly associated with the risk of incident cognitive decline. (B) Biological pathways with statistical overrepresentation in the predictive proteins for cognitive decline (p-value < .05, q-value < .2) for cognitive decline. (C) Volcano plot of significant proteins for progression from CIND to dementia. CNID, cognitive impairment no dementia.

(CTSB), Beta-glucuronidase (GUSB), insulin-like growth factor-binding protein 2 (IGFBP2), corticosteroid 11-beta-dehydrogenase isozyme 1 (HSD11B1), and WAP, Kazal, Immunoglobulin, Kunitz and NTR domain-containing protein 2 (WFIKKN2) (Figure S5A, Table S13). The top 10 proteins associated with a steeper decline in CDR-SB scores over time in each cognitive subgroup are visualized as volcano plots in Figure S5B. Within each cognitive subgroup, diverse biological pathways, including those related to cell adhesion, immune dysregulation, and inflammation, were overrepresented in the plasma proteomic profile linked to a steeper decline in cognitive function over time. The top five biological pathways for each cognitive subgroup are presented in Figure S5C, with all overrepresented pathways presented in Table S14.

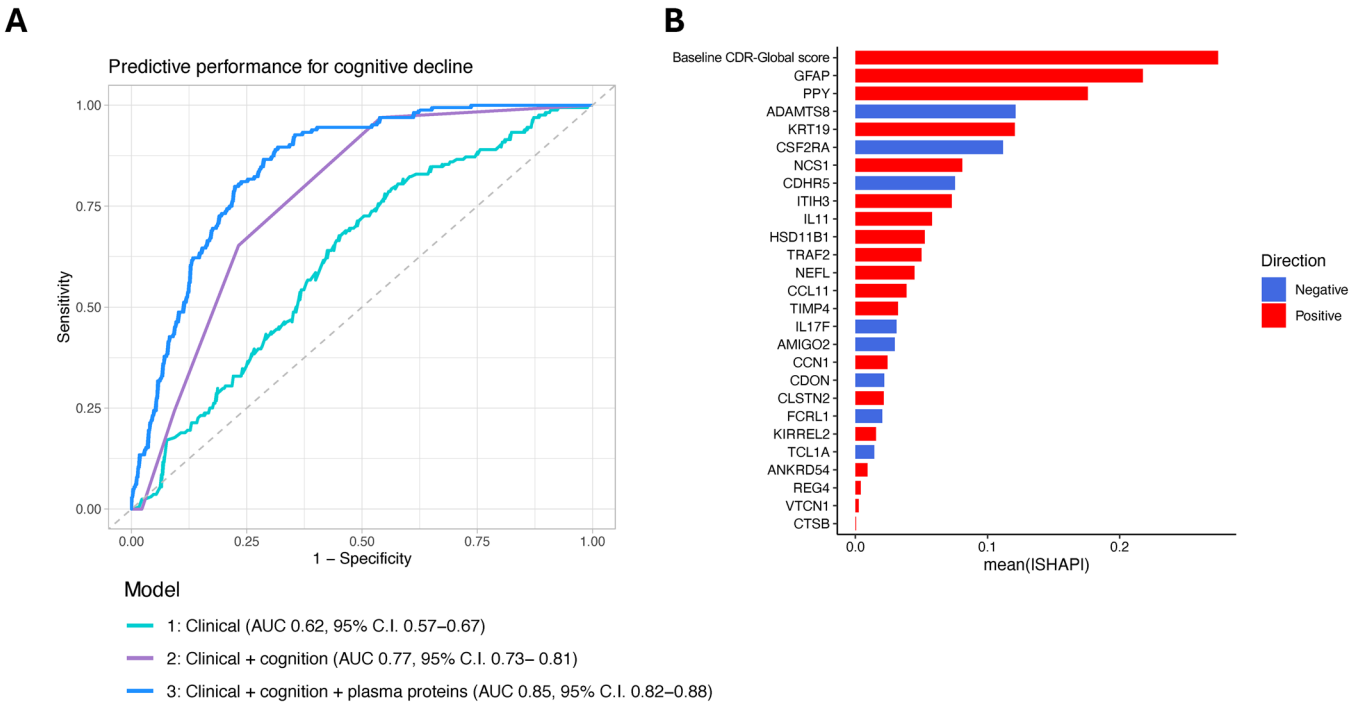

FIGURE 3 (A) Receiver-operating characteristic (ROC) curves of the predictive models with clinical risk factors, cognition, and plasma proteins. (B) Feature importance plot of the selected clinical risk factors and proteins. Abbreviations: AUC, area under the ROC curve; ROC, receiver-operating characteristic; SHAP, Shapley additive values.

TABLE 3 Ten plasma proteins for the prediction of progression from CIND to incident dementia.

Assay	Description	aHR[a]	95% C.I., LL	95% C.I., UL	*p*-value	*q*-value	C-index
AREG	Amphiregulin	2.70	1.88	3.89	9.24E-08	6.25E-05	0.719
NEFL	Neurofilament light polypeptide	2.17	1.63	2.88	1.04E-07	6.25E-05	0.756
TACSTD2	Tumor-associated calcium signal transducer 2	6.92	3.00	16.00	5.96E-06	2.39E-03	0.757
GFAP	Glial fibrillary acidic protein	2.34	1.58	3.46	2.27E-05	5.82E-03	0.744
MDK	Midkine	2.01	1.45	2.77	2.43E-05	5.82E-03	0.707
KLK4	Kallikrein-4	1.67	1.28	2	1.35E-04	2.69E-02	0.707
MUC13	Mucin-13	2.38	1.50	3.78	2.19E-04	3.45E-02	0.707
RSPO1	R-spondin-1	3.11	1.70	5.69	2.30E-04	3.45E-02	0.728
PIGR	Polymeric immunoglobulin receptor	3.91	1.88	8.15	2.65E-04	3.53E-02	0.712
CHGB	Secretogranin-1	2.34	1.48	3.71	2.97E-04	3.56E-02	0.706

Abbreviations: aHR, adjusted hazard ratios; *APOE* 4, apolipoprotein E ε4 allele; CDR, Clinical Dementia Rating; CIND, cognitive impairment no dementia.
[a]Adjusted hazard ratios (aHRs) and 95% confidence intervals (C.I.s) were derived from Cox proportional hazards regression adjusted for age, gender, hypertension, hyperlipidemia, diabetes, years of education, antihypertensive medication, lipid-lowering medication, obesity, and *APOE* ε4 status. Predictive power of individual proteins was assessed using Harrel's C-index. Type 1 error rates with multiple testing correction were estimated using *q*-values.

3.3 | Internal validation with quantitative immunoassays

Internal validation of Olink-measured biomarkers was performed using quantitative biomarker platforms. Paired, log10-transformed plasma levels of NEFL (SIMOA, $n = 45$), GDF-15 (Quantikine, $n = 33$), and NT-proBNP (Roche Elecsys, $n = 33$) were significantly correlated with their Olink-measured counterparts (R 0.77–0.82, $p < .001$; Figure 4A).

3.4 | External validation in the CSF proteome of the ADNI cohort

Of the 681 eligible subjects in the ADNI cohort (mean (± SD) age 73.3 ± 7.4 years, 57.3% male, 95.2% Caucasian, 24.2% NCI, 57.0% MCI, 18.8% dementia), 159 (23.3%) developed cognitive decline (ADNI subject demographics are presented in Table S15), over a mean (± SD) follow-up duration of 33.9 ± 14.2 months. Of note, the Somalogic platform used in the ADNI cohort did not include two (GUCA2A, NCS1)

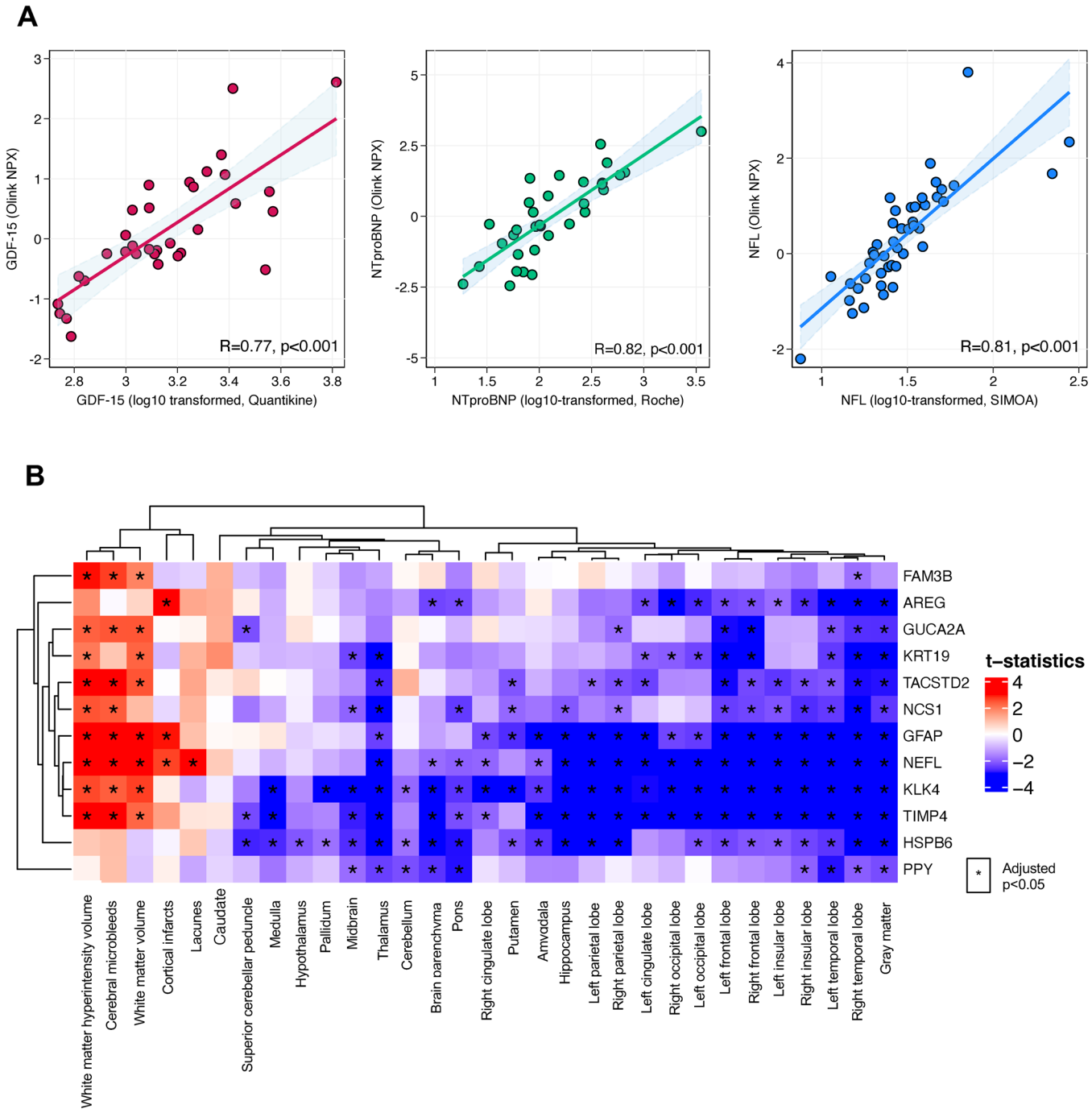

FIGURE 4 (A) Internal validation of Olink NPX values with paired quantitative immunoassays. (B) Heatmap of cross-sectional associations between 12 significantly predictive plasma proteins for cognitive decline and neuroimaging markers in the MACC cohort. MACC, Memory Aging and Cognition Centre; NPX, Normalized Protein eXpression.

of the 12 predictive plasma proteins identified for the MACC cohort (Olink platform).

Four proteins (AREG, PPY, NEFL, and GFAP) were concordantly associated with the risk of incident cognitive decline in the plasma proteome of the MACC cohort (Figure 5A) as well as the CSF proteome of the ADNI cohort (Figure 5B, Table S16). All four cross-validated proteins are associated with an increased hazard of cognitive decline (adjusted hazard ratio [aHR] >1).

3.5 | Associations of significantly predictive plasma proteins with neuroimaging markers of CSVD and brain volume

Cross-sectional analysis of the 12 significantly predictive baseline plasma proteins with structural neuroimaging markers of CSVD and brain volumetric indices of the MACC cohort are presented as a heatmap in Figure 4B. All 12 proteins are associated with one or more

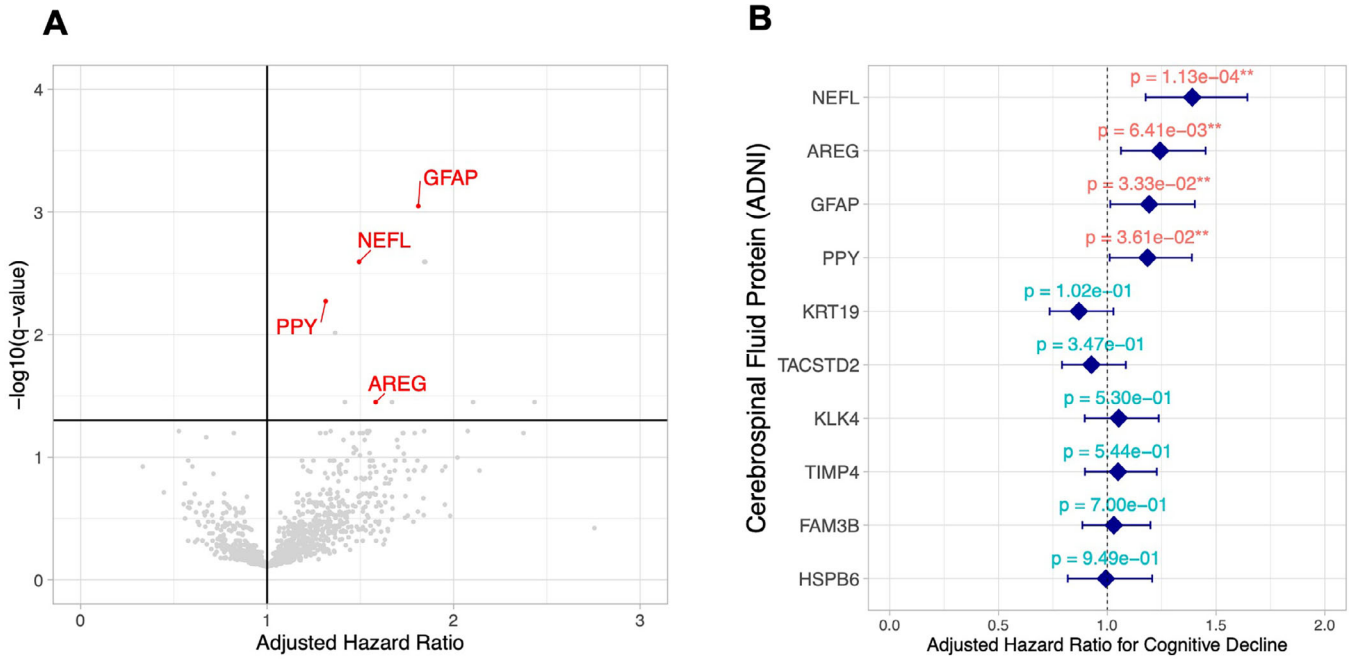

FIGURE 5 External validation of findings (A, B). Volcano and Forest plots of concordantly cross-validated significant predictive proteins for cognitive decline in: (A) the plasma proteome of the MACC cohort and (B) the CSF proteome of the ADNI cohort. ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; MACC, Memory Aging and Cognition Centre.

markers of CSVD or regional brain volumetric indices. Of these, 10 proteins (AREG, protein FAM3B [FAM3B], GUCA2A, KRT19, TACSTD2, NCS1, GFAP, NEFL, KLK4, and TIMP4) showed significant associations with both CSVD and brain volumetric markers.

4 | DISCUSSION

Using plasma proteomic analysis, we identified prognostic protein signatures of incident dementia and cognitive decline in a Southeast Asian cohort with high CeVD burden. These proteins related closely to neuroimaging markers of neurodegeneration and CSVD. The plasma proteins notably provided significant incremental value to clinical factors and baseline cognition for the prediction of future cognitive outcomes. Diverse biological pathways overexpressed in association with the protein profile linked to cognitive decline, related to inflammation, extracellular matrix disruption, immune dysregulation, and the regulation of the IGFBP axis. AREG, GFAP, NEFL, and PPY were externally validated as prognostic proteomic markers for cognitive decline, concordantly altered within the CSF proteome of an independent validation cohort.

Several of our top prognostic biomarker candidates for cognitive decline and conversion to dementia (NEFL, TIMP4, GFAP, and PPY) have been associated previously with dementia-related cognitive outcomes.[33–36] A case–control study comprising 60 individuals with MCI reported NEFL and TIMP4 as significant Olink biomarkers for cognitive decline.[16] However, GFAP was not included in that study.[16] Our results are also consistent with longitudinal studies from the UK Biobank evaluating the prognostic ability of Olink plasma proteins for incident dementia over 15 years in a predominantly community-based Caucasian cohort. Several top prognostic markers reported within the UK Biobank study, including GFAP, NEFL, TIMP4, and NCS1, were similarly replicated within our cohort.[13,37] Our findings therefore highlight the prognostic relevance of these biomarkers, which appear related to axonal degeneration, neuroinflammation, cardio-inflammatory processes, and neuronal calcium signaling, even in later-stage disease phenotypes over medium-term follow-up.[34,38,39] Of interest, several top biomarkers, such as GDF-15 and LTBP2, which predicted 15-year incident dementia in the UK Biobank, were not among the top prognostic proteins within our cohort.[13] This may reflect the potentially stage-specific nature of dementia biomarkers.[17] It is plausible that senescence-associated markers such as GDF-15 and LTBP2 may play a greater mid-life prognostic role for later-life cognitive decline as a biomarker of accelerated aging phenotypes.[40–42] However, compared with older subjects with an elevated concomitant burden of age-related pathology, we hypothesize that biomarkers specific to neuronal injury may more directly reflect the extent of neuropathological insult and better risk stratify for medium-term cognitive outcomes. Nonetheless, our findings extend current knowledge, reinforcing the prognostic utility of neuronal injury biomarkers for the prognostication of cognitive decline, even in later-stage elderly subjects.

Our top prognostic markers, namely NEFL and GFAP, differ from the top candidates identified in several studies utilizing the Somalogic platform for profiling the circulating proteome. A large population-based study incorporating the Invecchiare in Chianti, Baltimore Longitudinal Study of Aging (BLSA), and Religious Orders Study cohorts found MSTN, PI3, TFF3, and PAPPA to be associated with cognitive decline.[10] The Atherosclerosis Risk in Communities (ARIC) and

Whitehall-II cohorts found NTproBNP and CDCP-1 to be the most significantly predictive proteins for incident dementia over 20 years.[11] The BLSA identified mid-life levels of plasma GDF-15 to be most strongly associated with cognitive decline, whereas the ARIC study identified Sushi, EGF, SVEP1, WFDC2, AGRP, and NTproBNP as the top incident dementia-associated proteins over 20 years.[12,14]

This inter-study variation in the identification of the top prognostic markers may be attributed to several factors. First, there are between-study differences in the breadth of proteomic analytic coverage.[43] For example, there are inter-platform differences in coverage by Somalogic compared to Olink.[43,44] These proteomic screening platforms may also produce some non-quantitative results that correlate poorly with gold standard enzyme-linked immunosorbent assays (ELISAs). A recent study reported discordant correlations of circulating NEFL levels quantified using Somalogic and quantitative SIMOA immunoassays.[44] In contrast, the same study reported good correlations between circulating NEFL levels measured with Olink and SIMOA.[44] Our internal validation similarly found strong correlations between paired circulating NEFL levels measured using Olink and SIMOA, aligning with the current literature and reinforcing the validity of conclusions drawn.[44] Of interest, in the CSF, where NEFL is in greater abundance, good correlations have been reported between NEFL levels measured by Somalogic and quantitative immunoassays.[45] This is consistent with our observation of concordant upregulation of NEFL anticipating cognitive decline, both by Olink assay of plasma in the primary cohort, and by Somalogic aptamer-based assay of CSF in the external validation cohort. Finally, it is plausible that variation in the rankings of prognostic proteins may be related to inter-study differences in subject characteristics, follow-up time, and the potentially stage-specific nature of dementia biomarkers.[17] Indeed, our secondary analyses prioritized unique and overlapping proteins across the different cognitive subgroups, highlighting the importance of evaluating disease stage-specific biomarkers of longitudinal cognitive outcomes.

Among the significant biological pathways linked to the protein profiles associated with cognitive decline, 10 of 16 identified pathways related to inflammation and immune dysregulation. This is unsurprising, considering current evidence linking inflammation and immune dysregulation with the progression of cognitive impairment.[46–48] An identified biological pathway for cognitive decline related to the regulation of IGF transport and IGFBPs. This fits with the emerging role of the IGF superfamily as neurotrophic factors, contributing to neurogenesis, cell survival, and neuronal homeostasis.[49–51] Our findings are consistent with previous clinical studies that have demonstrated significant associations of the IGFBP superfamily with cognitive and functional outcomes.[52,53]

Diverse pathophysiological processes were suggested by the 12-protein plasma prognostic signature for cognitive decline. These included axonal injury or neuroinflammation (AREG, TIMP4, GFAP, and NEFL), extracellular matrix disruption (GFAP and KLK4), axonal regeneration (NCS1), signal transduction (GUCA2A), growth factor receptors (TACSTD2), apoptosis (FAM3B and HSPB6), notch signaling (KRT19), and gastrointestinal neuropeptide signaling in the gut–brain axis (PPY).[54–59] On a neuropathological level, this was likely underpinned by CSVD or neurodegenerative etiology, evidenced by the significant associations of these proteins with a broad range of neuroimaging markers. Of these, four proteins (GFAP, PPY, AREG, and NEFL) were additionally cross-validated for their association with cognitive decline in the CSF proteome of an independent cohort. Evidence regarding the prognostic utility of AREG, a novel protein candidate of pathobiological relevance to cognitive decline and one of the most significantly predictive proteins for cognitive outcomes within our cohort, is comparatively limited and hence discussed in further detail. AREG (Amphiregulin) is a ligand for the epidermal growth factor receptor and thought to suppress interleukin (IL)-6 secretion from microglia and astrocytes.[60] It is hypothesized to be expressed in response to neuroinflammation by brain regulatory T cells, thus reflecting neuroinflammatory burden within the central nervous system.[60] Although AREG was found to be upregulated in acute in vivo stroke models, studies evaluating its prognostic utility for dementia have yielded conflicting results.[56] One study demonstrated its significant mediation of age associations with dementia, whereas another reported that it did not significantly mediate the relationship between *APOE* and dementia.[61,62] In addition to AREG's prognostic value for cognitive decline in the plasma proteome of the Southeast Asian memory clinic cohort, we also demonstrate replication of this evidence in the CSF proteome of an external Caucasian replication cohort. Taken together, these findings reinforce the need for future studies to further evaluate the prognostic and therapeutic utility of these emerging biomarkers for cognitive decline.

5 | LIMITATIONS

First, the proteins studied were obtained from commercially available, preselected panels. This carries with it the inherent biases of preselected panels, which may simply reflect the inevitable inter-antigen variability in effective assay signaling—which will also vary between platforms. This in turn results in differing coverage between proteomic platforms, potentially leading to differing conclusions.[43] Second, as one of the first studies investigating plasma proteomic biomarkers of cognitive decline in a Southeast Asian cohort with high prevalence of CeVD, our results are exploratory and should be validated in other cohorts. Nonetheless, our incident dementia and cognitive decline rates are consistent with previously reported rates in similar cohorts, which strengthens the generalizability of our results.[63,64] In addition, we have demonstrated the concordant dysregulation of several top plasma protein candidates for cognitive decline in the primary cohort (GFAP, NEFL, PPY, and AREG), in the CSF proteome of an external cohort. This corroboration reinforces the pathophysiologic relevance of these markers, which exhibit dysregulation in both the circulation and central nervous system. Taken together, this highlights their potential as mechanistic targets in the pathogenesis of cognitive decline.

6 | CONCLUSIONS

Our study identifies a prognostic plasma proteomic signature underpinning cognitive decline and incident dementia in a Southeast Asian cohort characterized by a high prevalence of CeVD. External

validation within the CSF proteome of an independent Caucasian cohort additionally replicated AREG, PPY, GFAP and NEFL as prognostic biomarkers for cognitive decline, showing concordance across both biofluids. Future mechanistic studies are required to elucidate the roles of these proteins as therapeutic targets for dementia and cognitive decline.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

Written informed consent was obtained from all human subjects.

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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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