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RESEARCH ARTICLE

A blood-based multi-pathway biomarker assay for early detection and staging of Alzheimer's disease across ethnic groups

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Abstract

INTRODUCTION: Existing blood-based biomarkers for Alzheimer's disease (AD) mainly focus on its pathological features. However, studies on blood-based biomark-

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ers associated with other biological processes for a comprehensive evaluation of AD status are limited.

METHODS: We developed a blood-based, multiplex biomarker assay for AD that measures the levels of 21 proteins involved in multiple biological pathways. We evaluated the assay's performance for classifying AD and indicating AD-related endophenotypes in three independent cohorts from Chinese or European-descent populations.

RESULTS: The 21-protein assay accurately classified AD (area under the receiver operating characteristic curve [AUC] = 0.9407 to 0.9867) and mild cognitive impairment (MCI; AUC = 0.8434 to 0.8945) while also indicating brain amyloid pathology. Moreover, the assay simultaneously evaluated the changes of five biological processes in individuals and revealed the ethnic-specific dysregulations of biological processes upon AD progression.

DISCUSSION: This study demonstrated the utility of a blood-based, multi-pathway biomarker assay for early screening and staging of AD, providing insights for patient stratification and precision medicine.

KEYWORDS
Alzheimer's disease, amyloid pathology, blood biomarkers, disease staging, early detection, patient stratification, precision medicine

HIGHLIGHTS

- The authors developed a blood-based biomarker assay for Alzheimer's disease.
- The 21-protein assay classifies AD/MCI and indicates brain amyloid pathology.
- The 21-protein assay can simultaneously assess activities of five biological processes.
- Ethnic-specific dysregulations of biological processes in AD were revealed.

1 | BACKGROUND

Early screening and classification of Alzheimer's disease (AD) are important for the management and intervention of the disease. In particular, the most recently developed drugs targeting beta-amyloid (Aβ), such as lecanemab, as well as other drug candidates in the development pipeline, target individuals with early-stage AD or mild cognitive impairment (MCI).[1–4] The discovery of the blood-based amyloid, tau, and neurodegeneration (ATN) biomarkers of AD—plasma Aβ42/40 ratio, tau/phosphorylated tau (p-Tau), and neurofilament light polypeptide (NfL)—raises the possibility of developing a blood-based test for the early detection of AD. Compared to other biomarker assays, such as brain imaging by positron emission tomography (PET) or protein measurement in cerebrospinal fluid (CSF), such a blood biomarker test would be simpler, less expensive, and less invasive, making it more feasible for population-scale AD screening. Specifically, plasma p-Tau181, p-Tau217, and p-Tau231 levels can accurately classify AD and are positively associated with Aβ deposition and tau phosphorylation in the brain.[5–7] Furthermore, plasma NfL level is associated with cognitive decline and neurodegeneration.[8,9] Nonetheless, these AD blood biomarkers mainly capture ATN-related pathological changes in diseased brains. Recent advances in ultrasensitive, high-throughput protein measurement technologies suggest that more than 7000 proteins are detectable in human blood—many of which are involved in different biological functions, such as inflammation and vascular functions.[10,11] The dysregulation of these non-ATN biological processes is associated with AD progression and participates in the pathogenesis of AD.[12–16] Therefore, a blood-based biomarker assay that captures the status of biological processes beyond those related to the ATN biomarkers is needed to examine the progression of AD more comprehensively. Such an assay will result in a more accurate AD diagnosis and facilitate therapeutic development for personalized treatment.

We previously conducted large-scale plasma proteomic profiling of AD by ultrasensitive proteomic assay and identified hundreds of AD-associated blood protein biomarkers.[13] These AD-associated blood biomarkers form clusters and are involved in different biological pathways. Moreover, changes in the levels of these blood biomarkers are associated with AD progression and stage. By adopting a machine learning-based mathematical model, we integrated the

level changes of these blood biomarkers to develop a scoring system for AD classification that can better exploit the predictive value of each protein biomarker.[13] Thus, our findings collectively suggest the feasibility of developing a blood-based biomarker assay capable of simultaneous examination of multiple biological activities in individuals as well as accurate and early disease detection and staging, much like what has started to emerge for less-accessible CSF biomarkers.[17,18]

Accordingly, in the present study, we developed an integrated proteomic assay for AD assessment by leveraging these AD-associated blood biomarkers involved in different biological processes. This blood-based biomarker assay consistently detected the level changes of 21 protein biomarkers in patients with AD or MCI in three independent cohorts of Chinese or European descent. Moreover, we adopted a machine-learning based–mathematical model to develop the AD risk scoring systems for populations of Chinese or European descent, which accurately distinguished patients with AD (area under the receiver operating characteristic curve [AUC] = 0.9407 to 0.9867) and patients with MCI (AUC = 0.8434 to 0.8945) from cognitively normal (CN) individuals in both ethnic groups. This blood-based assay also accurately staged AD and outperformed some plasma ATN biomarkers on indicating the development of Aβ pathology in the brain. In addition, this blood-based biomarker assay can simultaneously assess the activities of five biological processes—neurodegeneration, inflammation, innate immunity, vascular functions, and metabolic activities—to provide a multiscale physiological assessment of an individual's status in AD. Thus, we revealed the heterogeneity of AD progression between populations of Chinese and European descent, providing insights for patient stratification and the development of precision medicine. Taken together, we developed a high-performance, blood-based biomarker assay for AD that can serve as a powerful tool for early screening, classification, and staging of the disease in clinical settings.

RESEARCH IN CONTEXT

1. **Systematic review**: A literature review was conducted using traditional sources (eg, PubMed). While existing Alzheimer's disease (AD) blood biomarkers, including the blood amyloid/tau/neurodegeneration (ATN) biomarkers, mainly indicate the pathology of AD, evidence suggests that AD is associated with other non-ATN–related biological processes, including inflammation and vascular functions. Therefore, a blood-based assay capturing additional AD-associated biological processes is needed for the early detection and staging of AD, and comprehensive examination of disease status.
2. **Interpretation**: This study presents a blood-based, multipathway biomarker assay that accurately detects AD and indicates amyloid pathology. It demonstrates that a biomarker panel capturing multiple AD-associated biological processes can facilitate a comprehensive understanding of an individual's AD status, providing insights into patient stratification and precision medicine.
3. **Future directions**: The clinical applications of this assay will be bolstered by prospective longitudinal studies in diverse ethnic groups. Moreover, studies applying this assay to other neurodegenerative diseases will further demonstrate its specificity and versatility.

2 | METHODS

2.1 | Study design

In this observational study, we developed a blood-based biomarker assay for AD by integrating AD-associated blood protein biomarkers from different biological processes. We consecutively recruited three independent cohorts: (1) a total of 1000 Hong Kong Chinese participants as Hong Kong Chinese cohort_1 for the initial evaluation of the biomarker assay and establishment of the AD risk scoring system for AD; (2) 47 Hong Kong Chinese participants as Hong Kong Chinese cohort_2 for an independent observational study to validate the biomarker assay for AD and MCI classification as well as indicate AD-related pathological changes (eg, amyloid deposition); and (3) 217 participants of European descent from the Spanish BIODEGMAR cohort for an independent observational study for validation of the biomarker assay in a population of European descent. The demographic and phenotypic data of the three cohorts are summarized in Table 1. The investigators who performed protein detection and other related experiments were blinded to the phenotypes of the human participants.

2.2 | Participant recruitment and assessment

2.2.1 | Hong Kong Chinese cohorts

We recruited two independent cohorts from the Hong Kong Chinese population. Correspondingly, Hong Kong Chinese cohort_1 comprised 1000 Hong Kong Chinese individuals aged 60 years or older, including 493 individuals with AD, 190 individuals with MCI, and 317 CN individuals, who visited the Specialist Outpatient Department of the Prince of Wales Hospital of the Chinese University of Hong Kong (CUHK-PWH). Meanwhile, Hong Kong Chinese cohort_2 comprised 47 Hong Kong Chinese individuals aged 60 years or older, including 25 individuals with Aβ+AD, 13 with Aβ+MCI, and 9 Aβ−CN individuals, who visited the Division of Neurology of CUHK-PWH. Participants of both cohorts underwent clinical examination, the MoCA,[19] blood collection for the measurement of the plasma ATN biomarkers (ie, Aβ42/40 ratio, p-Tau, and NfL), and neuroimaging by magnetic resonance imaging (MRI). The participants of Hong Kong cohort_2 were further subjected to Aβ-PET using [^{11}C]-Pittsburgh Compound B (PiB).

TABLE 1 Demographic characteristics of the study cohorts.

Dataset 1	Data	CN	MCI	AD
Hong Kong Chinese cohort_1 (Chinese)	Sample size (n = 1000)	317	190	493
	MoCA score (SD)	25.17 (2.857)	20.15 (6.014)	12.70 (5.476)
	Plasma Aβ42/40 (SD)	0.058 (0.018)	0.058 (0.013)	0.048 (0.014)
	Plasma p-Tau181, pg/mL (SD)	1.880 (0.882)	2.739 (1.732)	4.798 (1.900)
	Plasma NfL, pg/mL (SD)	15.82 (5.059)	26.66 (16.04)	36.54 (21.24)
	Age, years (SD)	73.17 (4.643)	76.49 (7.302)	80.12 (6.334)
	Sex, male %	40.1%	37.9%	33.1%
Dataset 2	**Data**	**Aβ− CN**	**Aβ+ MCI**	**Aβ+ AD**
Hong Kong Chinese cohort_2 (Chinese)	Sample size (n = 47)	9	13	25
	MoCA score (SD)	27.89 (1.453)	23.69 (2.323)	14.00 (4.865)
	Global cortical-to-cerebellum [^{11}C]-PiB retention ratio, SUVR (SD)	1.278 (0.062)	1.614 (0.181)	1.672 (0.148)
	Global cortical-to-cerebellum [^{18}F]-T807 retention ratio, SUVR (SD)	1.008 (0.032)	1.118 (0.097)	1.268 (0.205)
	Plasma Aβ42/40 (SD)	0.058 (0.015)	0.047 (0.006)	0.048 (0.011)
	Plasma p-Tau181, pg/mL (SD)	1.733 (0.617)	3.160 (1.605)	3.992 (1.289)
	Plasma NfL, pg/mL (SD)	19.48 (3.934)	27.18 (18.10)	36.23 (20.03)
	Age, years (SD)	69.00 (3.464)	71.54 (7.367)	72.12 (6.405)
	Sex, male %	66.7%	30.8%	32.0%
Dataset 3	**Data**	**Aβ− CN**	**Aβ+ MCI**	**Aβ+ AD**
Spanish BIODEGMAR cohort (European descent)	Sample size (n = 217)	20	68	129
	MMSE score (SD)	29.00 (0.918)	26.26 (2.027)	18.75 (3.462)
	CSF Aβ42/40 (SD)	0.095 (0.015)	0.051 (0.016)	0.045 (0.010)
	CSF t-Tau, ng/mL (SD)	0.332 (0.110)	0.547 (0.297)	0.723 (0.351)
	Age, years (SD)	68.70 (6.906)	73.43 (4.463)	73.67 (5.562)
	Sex, male %	55.0%	52.9%	38.8%

Abbreviations: Aβ, beta-amyloid; AD, Alzheimer's disease; CN, cognitively normal individuals; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NfL, neurofilament light polypeptide; PiB, Pittsburgh Compound B; p-Tau, phosphorylated tau; SD, standard deviation; SUVR, standardized uptake value ratio.

Participants with an Aβ-PET cortical-to-cerebellum standardized uptake value ratio (SUVR) > 1.4 were classified as Aβ+. The clinical diagnosis of AD was based on the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.[20] The diagnosis of MCI was based on clinical evaluation of cognition by a neurologist. Each participant's age, sex, body mass index (BMI), years of education, and medical history were recorded. To quantify gray matter and hippocampal volumes, we processed T1-weighted MRI images by AccuBrain IV1.2 (BrainNow Medical Technology). This study was approved by the Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee at CUHK-PWH and the Hong Kong University of Science and Technology. All participants or legal guardians of participants with advanced dementia provided written informed consent for study participation and sample collection.

2.2.2 | Spanish BIODEGMAR cohort

The Spanish BIODEGMAR cohort enrolled individuals visiting the Cognitive Decline and Movement Disorders Unit of Hospital del Mar, Barcelona, Spain.[21] The present study included 217 participants of the BIODEGMAR cohort, consisting of 129 individuals with Aβ+AD, 68 individuals with Aβ+MCI, and 20 Aβ−CN individuals. Participants donated a blood sample, underwent detailed neurological and neuropsychological evaluation, including the Mini-Mental State Examination (MMSE),[22] brain MRI, and lumbar puncture for CSF collection for Aβ and tau biomarker measurement. Diagnoses of AD and MCI were established by clinical evaluation and neuroimaging assessment according to the National Institute on Aging–Alzheimer's Association guidelines (NIA-AA, 2011).[23,24] Participants with a CSF Aβ42/40 ratio <0.062 were classified as Aβ+. Participants' age, sex, and years

of education were recorded. This study was approved by the Parc de Salut Mar Clinical Research Ethics Committee of Hospital del Mar. All participants or legal guardians of participants with advanced dementia provided written informed consent for study participation and sample collection.

2.3 | Plasma preparation from human blood samples

We collected whole blood samples (3 mL) from participants into K3EDTA tubes (VACUETTE) and centrifuged at 2000 × g for 15 minutes to separate the plasma from the cell pellet. We then collected, aliquoted, and stored the plasma samples at −80°C until protein measurement.

2.4 | Plasma and CSF protein measurements

To assess the 21 AD-associated blood proteins (Table S1), we measured the absolute plasma levels of the 21 proteins in 1 μL plasma by proximity extension assay (PEA) technology using a custom Olink Focus panel on an Olink Signature Q100 instrument at the Hong Kong Center for Neurodegenerative Diseases (HKCeND) in Hong Kong, China. To calculate the absolute levels of corresponding proteins in the samples, for each of the 21 proteins, we generated three calibrators with known concentrations of human recombinant proteins; during each experiment, we input the readings of each protein for the calibrators in triplicate, the negative controls in triplicate, and the assayed samples into a four-parameter logistic (4PL) curve. We measured four sample controls in triplicate during each experiment and calculated the intra- and inter-assay coefficients of variation (%CV). For quality control, we added internal controls, including incubation controls, detection controls, and extension controls. Only assays that passed quality control steps, had an intra-assay CV < 15%, had an inter-assay CV < 25%, and had measured levels in the range of lower and upper limits of quantification were considered valid and used for downstream analysis (Table S1).

To assess the ATN biomarkers, we measured the plasma Aβ42/40 ratio and NfL levels in 120 μL plasma using a Quanterix Neurology 4-Plex E Advantage Kit (103670) and measured plasma p-Tau181 level in 120 μL plasma with a Quanterix P-Tau181 Advantage V2 Kit (103714). We performed all Simoa assays on a Quanterix HD-X Automated Immunoassay analyzer at HKCeND. We measured the CSF Aβ42/40 ratio and t-Tau levels of the BIODEGMAR cohort by Fujirebio Lumipulse G600II assay (703380) at Laboratori de Referència de Catalunya in Barcelona, Spain.

2.5 | Association analysis of plasma proteins with AD and related endophenotypes

Prior to analysis, we transformed the plasma protein levels and AD-related endophenotype values by z-score normalization using the "normalize()" function in the R som package (v0.3-5.1). We then determined the associations between the normalized protein levels and clinical phenotypes (ie, AD or MCI vs CN), adjusting for age, sex, history of cardiovascular disease (ie, heart disease, hypertension, diabetes mellitus, and hyperlipidemia), and BMI using the following linear regression model:

$$\textit{Normalized protein level} \sim \beta_1 AD + \beta_2 MCI + \beta_3 Age + \beta_4 Sex + \beta_5 CVD + \beta_6 BMI + \varepsilon,$$

where β is the weighted coefficient for the corresponding factors and ε is the intercept of the linear equation. Similarly, the associations between normalized protein levels and AD-related endophenotypes were determined using the following linear regression model:

$$\textit{Normalized protein level} \sim \beta_1 \textit{ADrelated endophenotype} + \beta_2 Age + \beta_3 Sex + \beta_4 CVD + \beta_5 BMI + \varepsilon.$$

We considered plasma proteins with a false discovery rate (FDR)-adjusted p-value of <0.05 as being significantly associated with AD or AD-related endophenotypes.

2.6 | Calculation of AD risk scores based on the 21-protein biomarker assay

We adjusted the plasma levels of the 21 protein biomarkers for the effects of age and sex using the weighted coefficients determined from fitting protein level, age, and sex into a linear model using Hong Kong Chinese cohort_1. To account for potential effects of ethnic differences, we adjusted the plasma protein levels of the BIODEGMAR cohort using the weighted coefficients of age and sex quantified from that cohort. We then calculated individual AD risk scores using the following linear model that included the adjusted plasma levels of the 21 protein biomarkers:

$$\textit{Individual AD risk score} = \frac{1}{1 + e^{-(\sum \beta_i \textit{Adjusted plasma protein level}_i + \varepsilon)}},$$

where the weighted coefficient (β_i) and intercept (ε) were determined by fitting the adjusted protein levels and clinical phenotype into a logistic regression model.[25] We established AD risk levels on the basis of the distribution of the AD risk scores: Individuals with scores of <32, 32 to 72, or >72 were classified as having low, moderate, or high risk, respectively. We evaluated the accuracy of AD and MCI classifications using the 21-protein biomarker panel or plasma ATN biomarkers by calculating AUCs using the "auc()" function in the R pROC package (v1.18.0).

2.7 | Correlation analysis between candidate AD biomarkers and amyloid pathology

To determine the correlations of the 21-protein-based AD risk score, plasma ATN biomarkers, and MoCA scores as a function of Aβ-PET in

Hong Kong Chinese cohort_2, we first adjusted endophenotypes by age and sex. We adjusted MoCA scores by years of education in addition to age and sex. We then selected Aβ−CN individuals with a SUVR of ≤1.25 as the baseline reference. We used the means and standard deviations in the baseline reference group to convert individual AD risk scores and AD-related endophenotype values into z-scores. We visualized the change in z-score as a function of Aβ-PET SUVR using a locally estimated scatterplot smoothing (ie, loess) curve using the "ggplot()" and "stat_smooth()" functions in the R ggplot2 package (v3.4.0). Similarly, we determined the correlation of the 21-protein-based AD risk score and MMSE score with the CSF Aβ42/40 ratio in the BIODEGMAR cohort. We adjusted MMSE scores by years of education, age, and sex. Aβ−CN individuals with a CSF Aβ42/40 ratio of ≥0.11 served as the baseline reference to calculate z-scores, and the correlation between z-scores and CSF Aβ42/40 ratio was visualized using a loess curve.

2.8 | Multiscale assessment of biological processes in AD

We used the plasma levels of five proteins from the 21-protein biomarker assay—NEFL, PARP1, CD33, LIFR, and PPY—to indicate the activities of neurodegeneration, inflammation, innate immunity, vascular functions, and metabolic activities, respectively. To normalize the changes of the five blood proteins into a unified scale from 0 to 100, for each protein, the level of the 25th percentile of CN individuals served as the baseline reference (ie, score = 100) while the most highly dysregulated levels in AD served as the bottom line (ie, score = 0). We performed linear regression to normalize the protein levels to the unified scale, and the level at which the protein achieved >90% specificity for AD classification served as an anchor point, with the normalized score compulsorily set as 60. For data visualization, we generated radar plots containing the average scores of the five biological processes in AD, MCI, and CN individuals in each cohort using the "radarchart()" function in the R fmsb package (v0.7.5).

2.9 | Statistical analysis and data visualization

For the remaining statistical analyses that are not mentioned above, we determined the associations of candidate AD biomarkers (ie, Aβ-PET, tau-PET, CSF Aβ42, CSF Aβ42/40 ratio, and CSF t-Tau) and AD risk scores with clinical phenotypes and AD-related endophenotypes by linear regression, adjusting for age and sex. We generated all statistical plots using either the "ggplot()" function in the R ggplot2 package (v3.4.0) or GraphPad Prism (v9.0).

2.10 | Data and code availability

All statistical data associated with this study are contained in the main text or Supplementary Information. The consent forms signed by individual participants from the Hong Kong Chinese cohort_1 and Hong Kong Chinese cohort_2 state that the research content will be kept private under the supervision of the hospital and research team. Therefore, the phenotypic and proteomic data of individual participants will only be available and shared in formal collaborations. A review panel hosted at HKUST will process and review any applications for data sharing and project collaboration, and promptly notify applicants with the decision. Researchers may contact sklneurosci@ust.hk for details about data sharing and project collaboration related to the present study.

2.11 | Code availability

The code for our statistical analyses and data visualization are available on GitHub (https://github.com/yjiangah/Biomarker_study).

3 | RESULTS

3.1 | Development of an integrated proteomic assay to measure the 21 AD-associated blood proteins

While a biomarker panel of multiple AD-associated blood proteins can accurately classify and stage AD,[13,26–28] the blood concentrations of these protein biomarkers have broad ranges, making them difficult to measure accurately or simultaneously using conventional proteomic assays, such as enzyme-linked immunosorbent assay (ELISA). Therefore, in this study, we leveraged an ultrasensitive and multiplex proteomic assay—PEA[29]—to develop an integrated proteomic assay that can simultaneously measure multiple AD-associated blood protein biomarkers. We selected the following 21 AD-associated blood proteins from the protein clusters that we identified previously[13] and integrated them into a single assay: CASP3, CD33, CD84, EIF4B, EIF4EBP1, F2R, FAM3B, FGFBP1, KLK4, KLK14, LIFR, NEFL, NELL1, NUCB2, PARP1, PDGFC, PPY, PRDX5, PSME1, RBKS, and TNFSF14. These proteins are involved in biological processes including neurodegeneration, immune response, inflammation, metabolism, and cardiovascular functions (Table S1). This integrated proteomic assay achieved high detectability (ie, 100% above the lower limit of quantification), high specificity (ie, no cross-reactivity among proteins), and high precision (ie, inter- and intra-assay coefficients of variation of <10%) for the measurement of all 21 proteins in human blood (Table S1).

3.2 | Changes of the 21 AD-associated blood proteins are associated with AD and MCI

We subsequently examined the associations between the changes of these 21 blood proteins and AD, MCI, and AD-related endophenotypes. We used the integrated proteomic assay to measure the blood proteins in Hong Kong Chinese cohort_1, which comprised 493 patients with AD, 190 patients with MCI, and 317 CN individuals

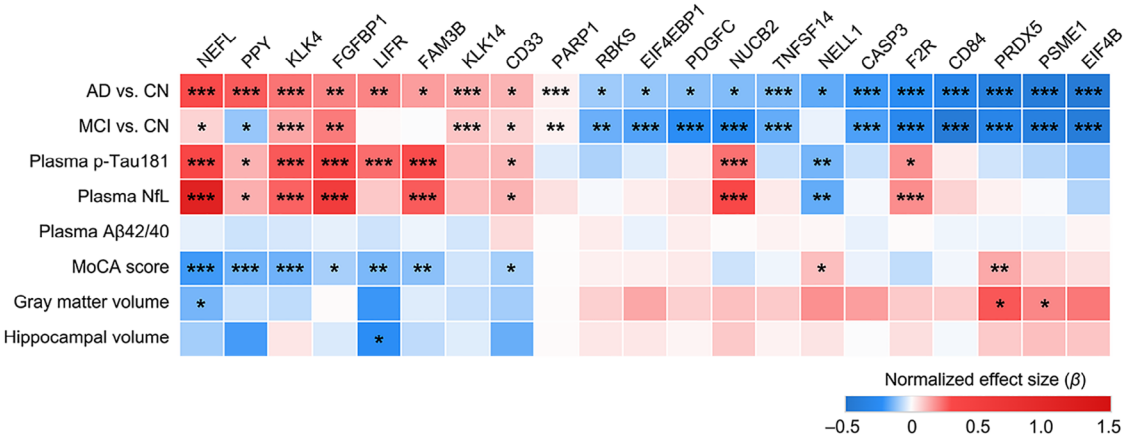

FIGURE 1 Associations between the levels of 21 plasma proteins and Alzheimer's disease (AD), mild cognitive impairment (MCI), and related endophenotypes. Heatmap showing the associations between the levels of 21 plasma proteins and AD, MCI, and related endophenotypes, including blood protein biomarker levels, cognitive performance, gray matter volume, and hippocampal volume (see Tables S2-S4 for details). Color intensity is proportional to the normalized effect size (β). Red and blue indicate positive ($\beta > 0$) and negative ($\beta < 0$) associations, respectively. Aβ, amyloid beta; CN, cognitively normal individuals; FDR, false discovery rate; MoCA, Montreal Cognitive Assessment; NfL, neurofilament light polypeptide; p-Tau, phosphorylated tau. *FDR < 0.05, ** FDR < 0.01, *** FDR < 0.001.

(Table 1). We then performed linear regression analysis to determine the relationships between the level of each protein and AD, MCI, and their related endophenotypes, adjusting for the effects of age, sex, status of cardiovascular diseases (ie, heart disease, hypertension, diabetes mellitus, and hyperlipidemia), and BMI. All 21 proteins were significantly dysregulated in AD plasma (Figure 1 and Table S2): nine proteins were upregulated (ie, NEFL, PPY, KLK4, FGFBP1, LIFR, FAM3B, KLK14, CD33, and PARP1), and 12 were downregulated (ie, EIF4B, PSME1, PRDX5, CD84, F2R, CASP3, NELL1, TNFSF14, NUCB2, PDGFC, EIF4EBP1, and RBKS). Moreover, the plasma levels of 18 of these blood proteins were altered in patients with MCI compared to CN individuals, suggesting that these proteins are dysregulated in early-stage AD (Figure 1 and Table S2). Furthermore, several blood proteins were associated with AD-related endophenotypes, namely the dysregulation of the ATN biomarkers in the blood (Figure 1, Figure S1, and Table S3), cognitive decline indicated by the MoCA score, and decreased volumes of gray matter and hippocampus (Figure 1 and Table S4). These results collectively suggest that the 21 blood protein biomarkers are associated with AD.

3.3 | An AD risk scoring system based on the 21-protein biomarker assay accurately classifies AD and MCI

Machine learning-based mathematical models that integrate the changes of multiple biomarkers can help better exploit the predictive values of each protein biomarker and accurately classify diseases.[25,30,31] Therefore, we applied this method to the 21-protein biomarker assay to establish an AD risk scoring system that generates an AD risk score for individuals based on the plasma levels of the 21 proteins (Figure 2A). Accordingly, in Hong Kong Chinese cohort_1, the AD risk scores accurately distinguished patients with AD (AUC = 0.9667) and patients with MCI (AUC = 0.8718) from CN individuals (Figure 2B–D). Moreover, as the plasma ATN biomarkers are the best-characterized and most widely studied blood protein candidates for AD classification,[32–34] we compared their performance for the classification of AD and MCI with that of the 21-protein biomarker assay in a subcohort of 357 participants. The results show that plasma NfL (AUC = 0.9177), plasma p-Tau181 (AUC = 0.9656), and the 21-protein biomarker assay (AUC = 0.9667) all distinguished patients with AD from CN individuals with >90% accuracy, whereas the plasma Aβ42/40 ratio had relatively low accuracy (AUC = 0.6654) (Figure 2C). However, when differentiating patients with MCI from CN individuals, only the 21-protein biomarker assay achieved >85% accuracy (AUC = 0.8718); meanwhile, plasma Aβ42/40 ratio (AUC = 0.5305), plasma NfL (AUC = 0.7790), and plasma p-Tau181 (AUC = 0.6510) achieved relatively low accuracy (Figure 2D). These results suggest that the 21-protein biomarker assay particularly outperforms these plasma ATN biomarkers for the detection of early-stage AD.

Given that specific proteins in the 21-protein biomarker assay are associated with AD-related endophenotypes (Figure 1), we examined whether this biomarker assay and the corresponding AD risk scoring system can be used to predict AD risk levels and associated endophenotypes. The distribution of AD risk scores suggests that most CN individuals, patients with MCI, and patients with AD had scores of <32, 32 to 72, and >72, respectively (Figure 2E). Therefore, we classified individuals with scores of <32, 32 to 72, or >72 as having a low, moderate, or high AD risk, respectively (sensitivity = 93.7% and 67.8%, specificity = 87.7% and 99.5% for cutoffs at 32 and 72, respectively; Table S5). Accordingly, the designated AD risk levels based on AD risk scores were significantly correlated with cognitive performance (β = −5.715, −9.369, and −3.654 for moderate vs low risk, high vs low risk, and high vs moderate risk, respectively; Figure 2F) as well as decreased hippocampal volume (β = −0.047, −0.080, and −0.033 for moderate vs low risk, high vs low risk, and high vs moderate

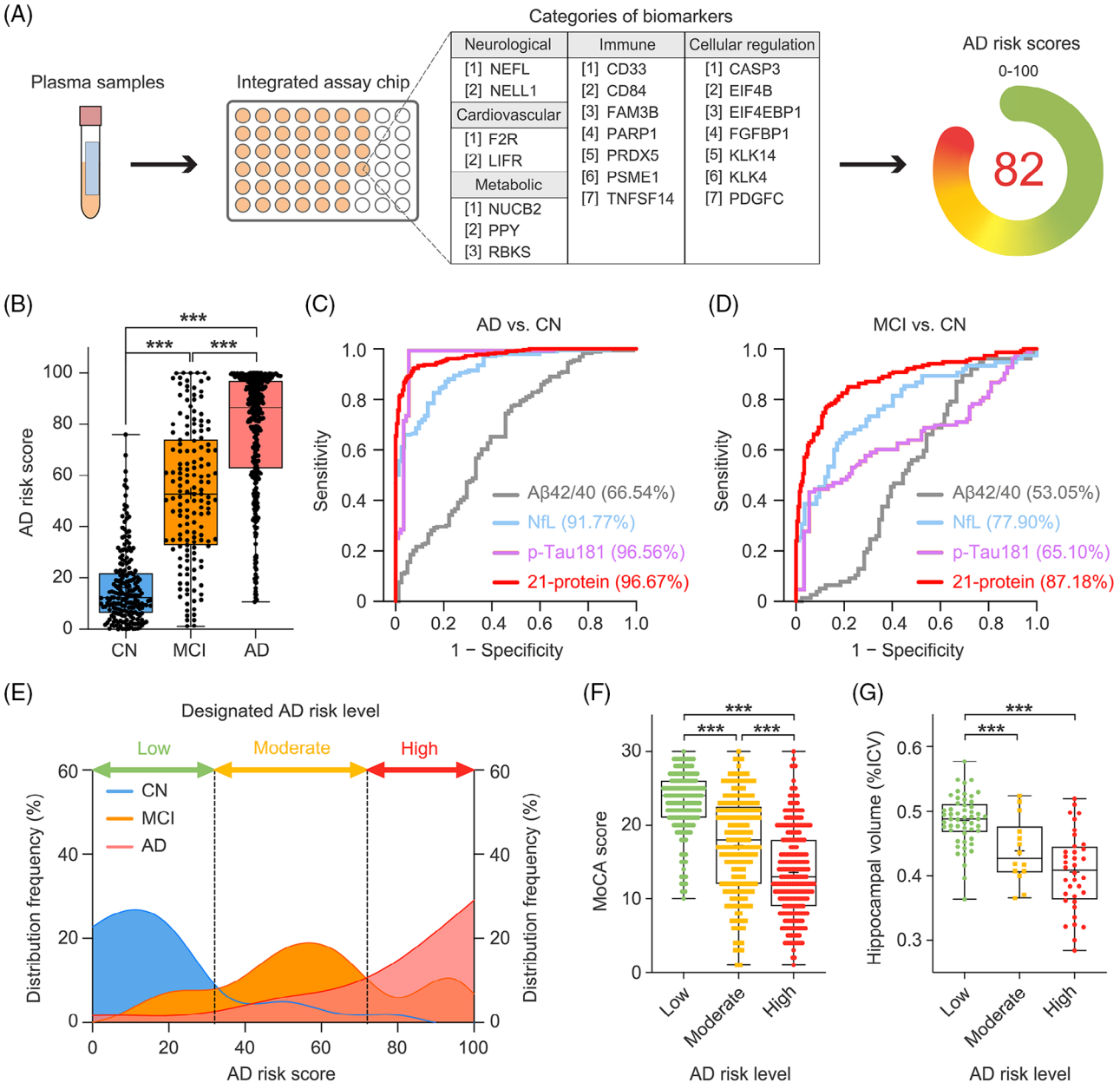

FIGURE 2 Development of the 21-protein biomarker assay and scoring system for the classification of Alzheimer's disease (AD) and mild cognitive impairment (MCI). (A) Schematic showing the calculation of AD risk scores for individuals based on the 21-protein biomarker assay. (B) Boxplot showing the individual AD risk scores in Hong Kong Chinese cohort_1 stratified by diagnosis ($n = 317$, 190, and 493 cognitively normal [CN] individuals, patients with MCI, and patients with AD, respectively; $\beta = 36.72$, 61.41, and 24.69 for MCI vs CN, AD vs CN, and AD vs MCI, respectively). (C,D) Receiver operating characteristic (ROC) curves showing the performance of the 21-protein biomarker panel and existing AD-associated blood biomarkers (ie, beta-amyloid [Aβ]42/40 ratio, neurofilament light polypeptide [NfL], and phosphorylated tau [p-Tau]181) for distinguishing patients with AD (C), or MCI (D), from CN individuals. Numbers in brackets indicate the area under the ROC (AUC), which indicate the model's performance in the corresponding classification. (E) Distribution of AD risk scores stratified by diagnosis. AD risk levels were categorized according to the distribution of AD risk scores: low risk, <32; moderate risk, 32 to 72; high risk, >72. (F) Individual cognitive performance indicated by Montreal Cognitive Assessment (MoCA) scores stratified by designated AD risk level ($n = 337$, 272, and 391 for low, moderate, and high risk, respectively; $\beta = -5.715$, −9.369, and −3.654 for moderate vs low risk, high vs low risk, and high vs moderate risk, respectively). (G) Individual hippocampal volumes stratified by designated AD risk level ($n = 53$, 14, and 36 for low, moderate, and high risk, respectively; $\beta = -0.047$, −0.080, and −0.033 for moderate vs low risk, high vs low risk, and high vs moderate risk, respectively). ICV, intracranial volume. Data in box-and-whisker plots include maximum, median, and minimum values as well as 25th and 75th percentiles; plus signs denote mean values. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

risk, respectively; Figure 2G). These results collectively demonstrate that the 21-protein biomarker assay not only accurately distinguishes patients with AD or MCI from CN individuals, but also indicates disease risk levels and AD stages. Therefore, this assay could serve as a powerful tool for AD screening and staging.

3.4 | The 21-protein biomarker assay accurately classifies AD and amyloid pathology in an independent Hong Kong Chinese cohort

The recent development of anti-amyloid drugs has spurred huge demand for a robust blood-based test for the early detection and monitoring of individuals with brain amyloid pathology.[1,2] Therefore, we subsequently examined the capability of our 21-protein biomarker assay to classify patients with amyloid pathology (ie, Aβ+ patients) and reflect the development of those AD-related pathologies. Specifically, we applied the 21-protein biomarker assay to an independent Hong Kong Chinese cohort_2, whose participants have undergone cognitive assessment (ie, MoCA) as well as amyloid-PET scan, tau-PET scan, and plasma ATN biomarker measurement (Table 1 and Figure S2). We adopted the same machine learning-based mathematical model established in the training dataset (ie, Hong Kong Chinese cohort_1) to calculate the AD risk score of individuals in this amyloid-PET–defined cohort. Consistent with the high performance of the biomarker assay in the training dataset, in Hong Kong Chinese cohort_2, the AD risk scores accurately distinguished patients with Aβ+AD (AUC = 0.9867) and patients with Aβ+MCI (AUC = 0.8945) from Aβ−CN individuals (Figure 3A–C). Again, this biomarker assay outperformed the plasma ATN biomarkers, particularly for detecting early-stage AD (ie, distinguishing patients with Aβ+MCI from Aβ−CN individuals; Figure 3B,C). Moreover, the AD risk scores generated by the model were negatively correlated with cognitive performance (r^2 = 0.1886; Figure 3D) and positively correlated with the development of amyloid pathology (r^2 = 0.3022; Figure 3E) and tau pathology (r^2 = 0.1397; Figure 3F) in the brain, corroborating the model's capability of indicating AD progression. Notably, we further showed that the 21-protein biomarker assay outperformed cognitive assessment by MoCA and the plasma ATN biomarkers when capturing the changes of amyloid pathology in the brain, as indicated by an earlier and stronger correlation with the amyloid-PET scan results (Figure 3G). Taken together, these results demonstrate that the 21-protein biomarker assay accurately detects amyloid pathology in the brain, demonstrating its potential for early screening, classification, and staging of AD as well as related pathological changes in clinical settings.

3.5 | Development of the 21-protein biomarker assay for the classification of AD and amyloid pathology in a population of European descent

Emerging blood proteomics studies suggest that blood proteins may have distinct baseline levels, patterns of dysregulation, and/or regulatory mechanisms among ethnic groups.[13,35–37] Therefore, it is important to establish ethnic-specific references, cutoffs, and models when developing blood biomarkers for AD.[35] Accordingly, we optimized our 21-protein biomarker assay specifically for the classification of AD and MCI in a population of European descent. We applied the 21-protein biomarker assay to the Spanish BIODEGMAR cohort,[21] whose participants have undergone cognitive assessment by MMSE as well as CSF measurement of the Aβ42/40 ratio and t-Tau (Table 1 and Figure S3). We then developed a European population-specific AD risk scoring system based on the level changes of the 21 AD-associated blood proteins in this cohort. The AD risk scores generated by this system adequately distinguished patients with Aβ+AD (AUC = 0.9407) and patients with Aβ+MCI (AUC = 0.8434) from Aβ−CN individuals (Figure 4A–C). Moreover, the AD risk scores were again negatively correlated with cognitive performance (r^2 = 0.2108; Figure 4D) and positively correlated with the development of amyloid pathology (r^2 = 0.1283; Figure 4E) and neurodegeneration (r^2 = 0.0564; Figure 4F) in the brain, as indicated by a decreased CSF Aβ42/40 ratio and increased CSF t-Tau level, respectively. Furthermore, the AD risk scores again better reflected amyloid pathology in the brain than cognitive assessment by MMSE score, as indicated by an earlier and stronger correlation with CSF Aβ42/40 ratio (Figure 4G). These results collectively demonstrate that our 21-protein biomarker assay, which integrates ethnic-specific models and scoring systems, can achieve early classification of AD and amyloid pathology in populations of Chinese or European descent.

3.6 | The 21-protein biomarker assay reveals distinct contributions of biological processes to AD progression in different ethnic populations

Besides its ability to accurately classify AD in both ethnic groups, the 21-protein biomarker assay also captured the heterogeneity of AD progression between the two populations in terms of the changes of biological processes. Specifically, we first compared the pattern of dysregulation of each protein among Hong Kong Chinese cohort_1, Hong Kong Chinese cohort_2, and the Spanish BIODEGMAR cohort. In the two Hong Kong Chinese cohorts, the 21 AD-associated blood proteins exhibited consistent dysregulation in patients with AD or MCI: All 21 proteins exhibited the same trends of changes in patients with Aβ+AD compared to Aβ−CN individuals (Figure S4A), and 18 of the 21 proteins (ie, all except KLK14, PPY, and PDGFC) exhibited the same trends of changes in patients with Aβ+MCI compared to Aβ−CN individuals (Figure S4B). This suggests that the 21-protein biomarker assay can consistently capture the protein signature changes in AD and MCI across different cohorts. Notably, in the population of European descent, six proteins (ie, RBKS, PDGFC, NUCB2, TNFSF14, CASP3, and F2R) exhibited the opposite trends of changes in Aβ+AD compared to those in the Chinese cohorts (Figure S4A). Moreover, eight proteins (ie, FGFBP1, PPY, RBKS, TNFSF14, CASP3, PDGFC, NUCB2, and F2R) also did not show consistent trends of changes in Aβ+MCI plasma between the population of European descent and the Chinese cohorts (Figure S4B). Thus, these data suggest that the 21-protein biomarker assay

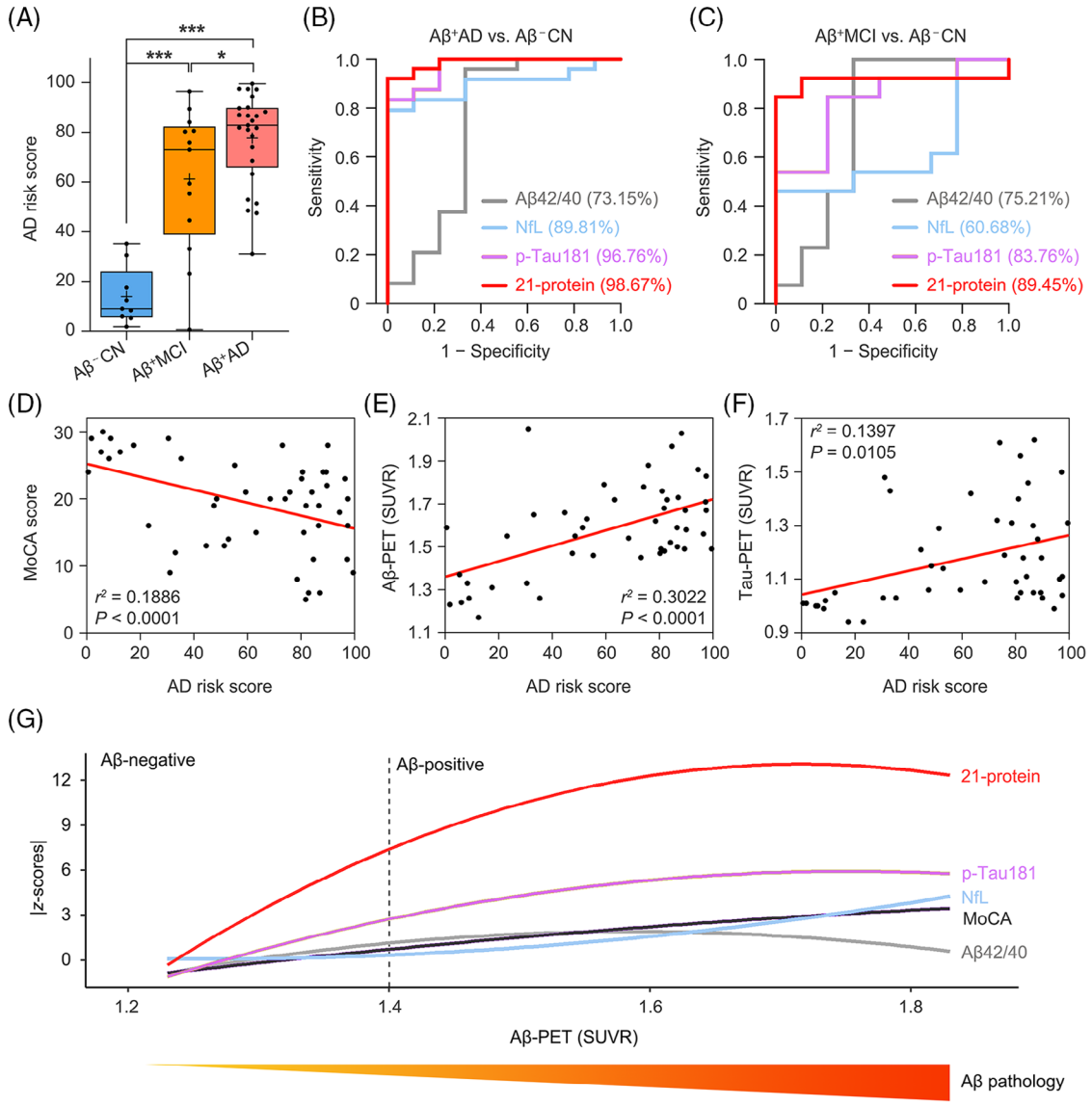

FIGURE 3 The 21-protein biomarker assay accurately classifies Alzheimer's disease (AD) and amyloid pathology in an independent Hong Kong Chinese cohort. (A) Boxplot showing the individual AD risk scores in Hong Kong Chinese cohort_2 stratified by diagnosis ($n = 9$ beta-amyloid [Aβ]− cognitively normal [CN] individuals, 13 Aβ+ patients with mild cognitive impairment [MCI], and 25 Aβ+ patients with AD; $\beta = 47.18$, 63.66, and 16.47 for Aβ+MCI vs Aβ−CN, Aβ+AD vs Aβ−CN, and Aβ+AD vs Aβ+MCI, respectively). Data in box-and-whisker plots include maximum, median, and minimum values as well as 25th and 75th percentiles; plus signs denote mean values. (B,C) Receiver operating characteristic (ROC) curves showing the performance of the 21-protein biomarker panel and existing AD-associated blood biomarkers (ie, plasma Aβ42/40 ratio, neurofilament light polypeptide [NfL], and phosphorylated tau [p-Tau]181) for differentiating patients with Aβ+AD (B), and Aβ+MCI (C), from Aβ−CN individuals. (D–F) Correlations between individual AD risk scores and AD-related endophenotypes, including cognitive performance indicated by Montreal Cognitive Assessment (MoCA) score (D), Aβ pathology in the brain indicated by amyloid-PET in global cortical regions (E), and tau pathology in the brain indicated by tau-positron emission tomography [PET] in global cortical regions (F). (G) Correlations between the progression of Aβ pathology in the brain and AD risk scores, AD-associated blood biomarkers, and cognitive performance indicated by MoCA score. r^2, Pearson's correlation coefficient; SUVR, standardized uptake value ratio; |z-scores|, absolute values of z-scores. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

can also reveal differences in the dysregulation of the blood proteome between populations of Chinese and European descent.

Moreover, given that these 21 AD-associated blood proteins are involved in different biological pathways (Table S1), we subsequently examined whether they could reveal AD progression based on changes in biological processes in the two ethnic groups. Accordingly, we selected the following five blood proteins that indicate the activities of biological processes: NEFL for neurodegeneration,[32] PARP1 for inflammation,[38,39] CD33 for innate immunity,[40,41] LIFR for vascular functions,[13,42,43] and PPY for metabolic activities.[44–47] By normalizing the changes of these five blood proteins into a unified scale from 0 to 100, we established a multiscale system that can simultaneously

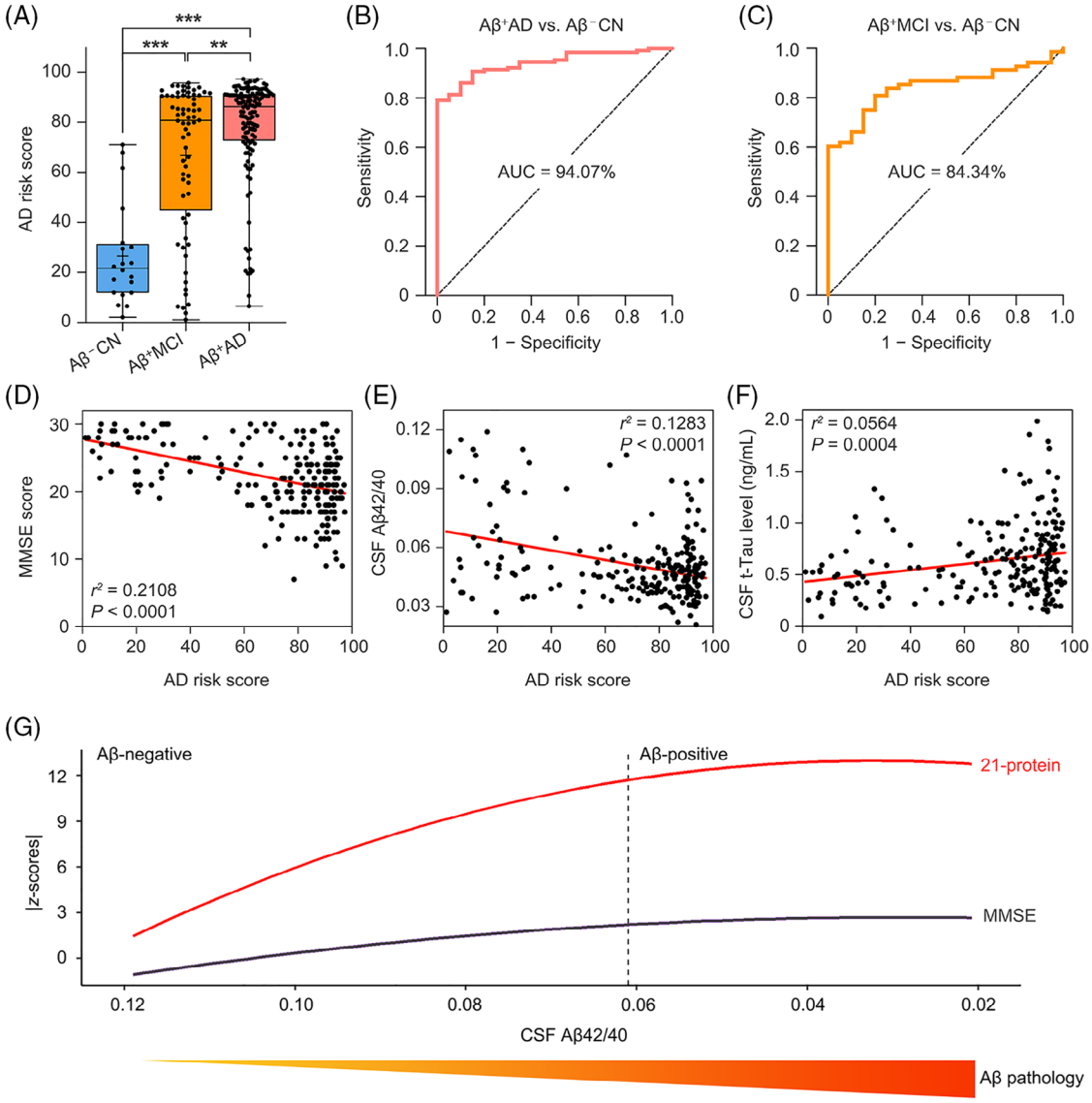

FIGURE 4 The 21-protein biomarker assay accurately classifies Alzheimer's disease (AD) and amyloid pathology in a population of European descent. (A) Boxplot showing the AD risk scores of individuals in the Spanish BIODEGMAR cohort stratified by diagnosis ($n = 20$ beta-amyloid [Aβ]− cognitively normal [CN] individuals, 68 Aβ+ patients with mild cognitive impairment [MCI], and 129 Aβ+ patients with AD; $\beta = 40.25$, 51.59, and 11.34 for Aβ+MCI vs Aβ−CN, Aβ+AD vs Aβ−CN, and Aβ+AD vs Aβ+MCI, respectively). Data in box-and-whisker plots include maximum, median, and minimum values as well as 25th and 75th percentiles; plus signs denote mean values. (B,C) Receiver operating characteristic (ROC) curves showing the performance of the 21-protein biomarker panel for differentiating patients with Aβ+AD (B), and Aβ+MCI (C), from Aβ−CN individuals. (D–F) Correlations between AD risk scores and AD-related endophenotypes of individuals, including cognitive performance indicated by Mini-Mental State Examination (MMSE) score (D), Aβ pathology in the brain indicated by cerebrospinal fluid (CSF) Aβ42/40 ratio (E), and neurodegeneration in the brain indicated by CSF t-Tau levels (F). (G) Correlations between the progression of Aβ pathology in the brain and AD risk score and cognitive performance indicated by MMSE score. r^2, Pearson's correlation coefficient; |z-scores|, absolute values of z-scores. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

evaluate the status of five biological processes in individuals. Accordingly, examination of the status changes of different biological processes in individuals revealed that the dysregulations of different biological processes exhibit distinct patterns upon AD progression: in both Hong Kong Chinese cohort_1 and cohort_2, the dysregulation of inflammation and innate immunity mainly starts in the early stage of AD (ie, MCI) and remains relatively constant throughout progression to AD (Figure 5A,B and Figure S5). Meanwhile, neurodegeneration continues developing during the development of MCI and AD, and the dysregulation of vascular functions and metabolic activities mainly occurs in the late stage (ie, AD) (Figure 5A,B and Figure S5). These results suggest that different biological processes may have stage-specific contributions to AD progression. Interestingly, when we examined the changes of individuals' biological processes in the population of European descent, the patterns of dysregulation of these biological processes were different from those in the Chinese populations. In the

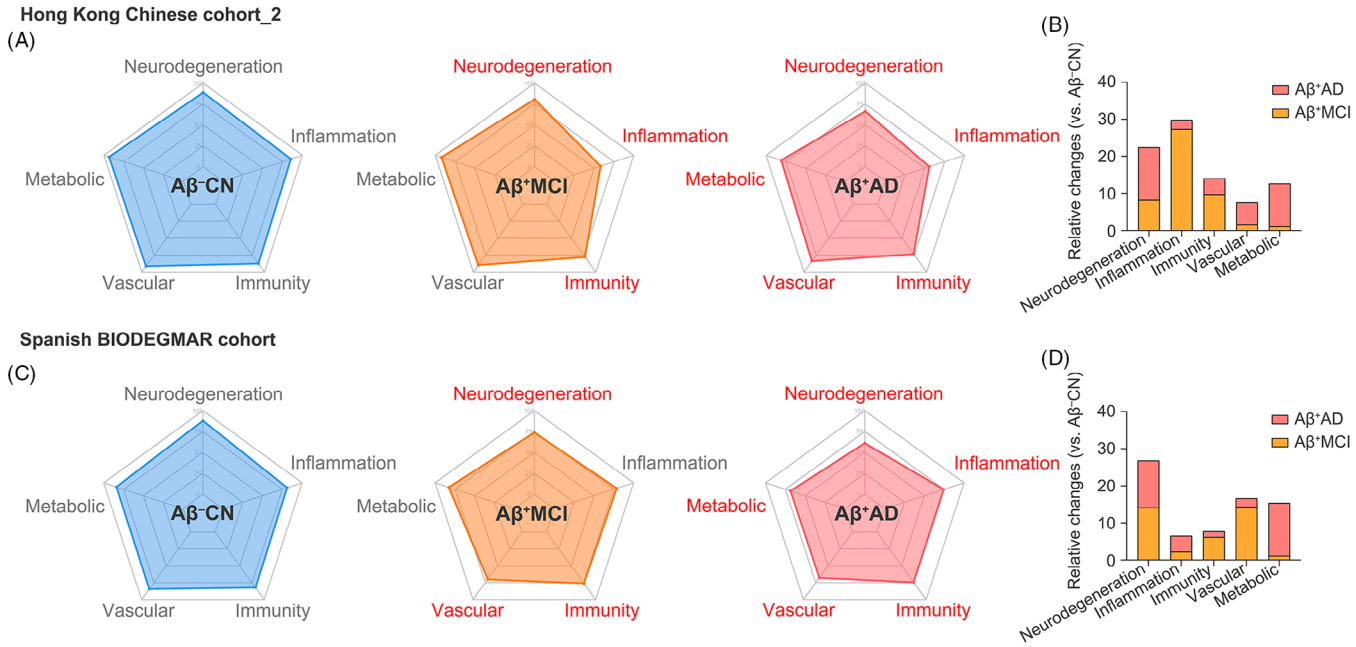

FIGURE 5 Dysregulation of different biological processes in Alzheimer's disease and mild cognitive impairment between populations of Chinese and European descent. Average scores (A,C) and relative changes compared to cognitively normal (CN) individuals (B,D) of five biological processes (ie, neurodegeneration, inflammation, innate immunity, vascular functions, and metabolic activities) in beta-amyloid (Aβ)-negative CN individuals (Aβ−CN), Aβ-positive patients with mild cognitive impairment (Aβ+MCI), and Aβ-positive patients with Alzheimer's disease (Aβ+AD) in Hong Kong Chinese cohort_2 (A,B), as well as Aβ−CN, Aβ+MCI, and Aβ+AD individuals in the Spanish BIODEGMAR cohort (C,D). The biological processes that are significantly dysregulated in patients with Aβ+MCI or Aβ+AD compared to Aβ−CN individuals are indicated in red. Immunity, innate immunity; Metabolic, metabolic activities; Vascular, vascular functions.

Spanish BIODEGMAR cohort, the dysregulation of vascular functions and innate immunity started at the early stage of AD (Figure 5C,D). Meanwhile, the dysregulation of inflammation and metabolic activities mainly occurred in the late stage (ie, AD) (Figure 5C,D). These findings suggest that the characteristics of the progression of MCI and AD might differ between populations of Chinese and European descent, particularly with respect to biological pathways related to inflammation and vascular functions. Taken together, our results demonstrate that the 21-protein biomarker assay captures the activities of multiple biological processes to reveal AD progression and its heterogeneity among ethnic groups. Therefore, it may facilitate a more comprehensive and detailed evaluation of the disease status of individuals and bolster therapeutic development for personalized treatment.

4 | DISCUSSION

A blood-based test for AD would be simple, objective, and noninvasive, making it advantageous for screening and monitoring of the disease.[48] Accordingly, in this study, we developed a proteomic assay to simultaneously measure the levels of 21 AD-associated blood proteins, which detects blood proteins with a broad concentration range (1.2×10^{-1} to 2.5×10^{6} pg/mL) and requires minimal sample input (<5 μL plasma). Moreover, we developed an AD risk scoring system that accurately classifies AD/MCI and amyloid pathology across ethnic groups based on changes in the level of these blood proteins in AD. Head-to-head comparison in an amyloid-PET–defined cohort demonstrated that our risk scoring system outperforms some existing blood ATN biomarkers for AD classification: The AD risk scores generated by the assay exhibit larger fold-changes upon the development of amyloid pathology (a 6.08-fold change for individuals with Aβ-PET, SUVR >1.8 vs <1.3) than the plasma Aβ42/40 ratio (0.74-fold), p-Tau181 (2.90-fold), or NfL (2.33-fold). Furthermore, in the population of European descent, the assay also accurately classified Aβ+AD and Aβ+MCI individuals, for which the performance is comparable to that previously reported for plasma p-Tau217 and p-Tau231.[7,49,50] Thus, our results collectively demonstrate that the 21-protein biomarker assay can be developed into a robust blood test for early screening and staging of AD across different ethnic populations. In addition, the unique ability of this assay to simultaneously capture the activities of multiple biological processes helps reveal the heterogeneity of AD progression among individuals/ethnic groups, which can facilitate the multi-pathway analysis of AD for patient stratification and provide insights for targeted treatment and intervention.

Early detection of AD, particularly for patients with amyloid pathology and early-stage cognitive symptoms (eg, those with Aβ+MCI) is important for effective intervention.[7,48] Lecanemab, an anti-amyloid monoclonal antibody recently approved by the US Food and Drug Administration for the treatment of early-stage AD, can significantly reduce brain amyloid burden and slow the rate of cognitive decline by 27% in Aβ+ individuals with mild dementia or MCI (average Clinical Demetia Rating-Sum of Boxes [CDR-SB] = 3.2).[1] Moreover,

donanemab, another anti-amyloid monoclonal antibody in phase III clinical trials, can slow cognitive decline by 35% versus placebo in those Aβ+ individuals with mild dementia or MCI (average MMSE = 22.9).[2,4] Of note, the individuals who are recommended to take these drugs[51] (ie, Aβ+ with MCI or mild dementia) have mild symptoms that are commonly overlooked in daily life. Consequently, many such individuals are diagnosed with AD only after severe cognitive impairments manifest, thereby missing the window for effective intervention and treatment.[52,53] Therefore, there is an urgent need for a diagnostic test for population-scale screening of individuals with early-stage AD. Moreover, as lecanemab and donanemab aim to reduce brain amyloid burden, the routine examination of Aβ pathological changes—to evaluate drug efficacy and optimize therapeutic strategy—should be simple and convenient. Notably, in addition to the ability to distinguish Aβ+MCI from healthy individuals, our 21-protein biomarker assay can also indicate the stages of AD, including cognitive decline, brain atrophy, and the development of brain amyloid pathology. Hence, this assay is a feasible solution for both participant prescreening as well as monitoring of disease stages and pathologies, and it can also support the evaluation of drug candidates in future clinical trials. Furthermore, compared to existing blood-based biomarker assays, this assay can evaluate health status more comprehensively by simultaneously assessing the activities of multiple biological processes. Therefore, it can provide additional information about the effects of anti-amyloid drugs on AD-associated biological processes beyond ATN-related pathologies, which may help differentiate the responders and nonresponders regarding clinical effects of the drugs.

Concurrent use of multiple biomarkers to determine disease status—termed as a "composite biomarker panel"—is an effective approach to exploit the predictive values of each biomarker candidate. Such biomarker panels are widely used to predict complex diseases such as heart disease and age-related diseases.[30,31] In AD, besides the well-known ATN-related pathologies, studies suggest that pathways related to innate immunity, inflammation, metabolism, and vascular functions are also associated with AD,[12,54–57] some of which become dysregulated at an early stage of AD and contribute to the pathogenesis of the disease.[12,54,55,57] For example, an increase of CD33, an immune molecule that regulates the activities of innate immunity in the blood and brain, is observed in AD[41,58] and is linked to both amyloid pathology and disease progression.[41] It is suggested that the increased blood level of CD33 plays a disease-causing role in AD pathogenesis.[40] In AD transgenic mouse model studies, CD33 slows Aβ clearance by microglia, and its deletion mitigates amyloid pathology in the brain.[41,59] These findings suggest that a dysregulated CD33 level, which leads to impaired innate immunity, may be an early-stage biomarker of AD—possibly even before the occurrence of amyloid pathology. Therefore, we anticipate that including these early-stage biomarkers in a blood test will aid the early detection of AD. Our results suggest that other blood biomarkers, such as FGFBP1 and CD84, exhibit altered levels in early-stage AD/MCI. Therefore, a more comprehensive screening of blood biomarkers for early-stage AD/MCI may help identify additional early biomarkers of AD and improve the performance of the blood test for early screening.

In addition, by measuring changes in the levels of blood biomarkers from different biological processes, we demonstrated that the 21-protein biomarker assay is capable of multi-pathway assessment of AD, thereby revealing the heterogeneity of AD progression among individuals and ethnic groups. This provides critical insights for patient stratification and precision medicine. Indeed, studies suggest that ethnicity is an important factor underlying the heterogeneity of AD.[60–63] For example, apolipoprotein E (*APOE*) ε4, the strongest known genetic risk factor for sporadic AD, is more frequent in African Americans (frequency = 0.267) than in populations of European descent (frequency = 0.155) and East Asian descent (frequency = 0.086)[60,61]; however, its AD risk effect is stronger in populations of East Asian descent (odds ratio = 5.6) than in other populations (odds ratio = 2.7 and 1.1 in populations of European descent and African Americans, respectively).[60] Moreover, baseline CSF levels of tau and p-Tau181 are higher in populations of European descent than in African Americans.[63] Furthermore, plasma NfL and plasma p-Tau181 are differentially modulated by comorbidities across ethnic groups.[64,65] Concordantly, our results show that patterns of the dysregulated blood proteome in AD differ between populations of Chinese and European descent, particularly in biological pathways related to inflammation or vascular functions. These findings collectively suggest that pathophysiological mechanisms and patterns of progression of AD are distinct in different ethnic groups, which highlights the importance of developing diagnostic assays and criteria for ethnic-specific disease assessment and treatment. Furthermore, pilot studies suggest that the presence of AD subtypes may also contribute to interindividual variability of phenotypes and progression of AD.[66–69] Particularly, transcriptome analysis of *post mortem* AD brains revealed that different AD subtypes with distinct molecular signatures of neuroinflammation, immune activity, mitochondria organization, and neurogenesis may differ with respect to Aβ plaque density and the degree of cognitive decline.[69] Interestingly, several genes, including *CD33*, *LIFR*, *NEFL*, and *NELL1*, were identified as the key drivers of those AD subtypes[69]; their blood protein levels are measured in our 21-protein biomarker assay. Therefore, it is worth investigating if these blood biomarkers and our assay can capture the specific brain signatures of AD subtypes, which may help stratify AD into subtypes and identify alternative pathological mechanisms of the disease.

Further studies and optimization of the 21-protein biomarker assay will aid its incorporation into clinical settings. First, longitudinal studies examining the performance of this assay to predict cognitive decline and AD risks would corroborate its utility for early prediction and monitoring of the disease. Second, although this assay can accurately distinguish Aβ+ individuals from Aβ−CN individuals, it is necessary to understand how well it can differentiate AD from non-AD dementias, including Parkinson's disease dementia, Lewy body dementia, frontotemporal dementia, and vascular dementia, which will aid the development of a highly specific AD blood-based test. Last, while this assay can simultaneously assess five biological processes, including biomarkers of additional biological pathways may better capture disease status. For example, several blood proteins, such as soluble ST2, are evidenced to have disease-causing effects in AD, which makes

them candidate drug targets for treatment.[12] Adding those blood proteins to the assay might facilitate patient screening and monitoring of treatment effectiveness. Such work will pave the way for a more comprehensive evaluation of AD through a blood-based test and provide insights for personalized care and therapeutics for the disease.

In summary, we have developed a blood-based biomarker assay comprising 21 proteins related to different biological pathways. We also established a highly accurate scoring system for the classification of AD and MCI across ethnic groups. Our findings demonstrate the feasibility of a blood-based biomarker assay for early screening and routine monitoring of pathological changes of AD. Moreover, the heterogeneity of AD progression between ethnic groups and individuals revealed by our assay emphasizes the importance of patient stratification and precision medicine for AD diagnostics and therapeutics.

AUTHOR CONTRIBUTIONS

Yuanbing Jiang, Fanny C. Ip, Amy K. Y. Fu, and Nancy Y. Ip conceived of the study; Fanny C. Ip, Ronnie M. N. Lo, Brian C. H. Law, Paula Ortiz-Romero, Albert Puig-Pijoan, Aida Fernández-Lebrero, José Contador, Timothy C. Y. Kwok, and Vincent C. T. Mok organized patient recruitment and sample collection; Yuanbing Jiang, Elaine Y. L. Cheng, Xiaoyun Cao, and Clara M. C. Tan performed the experiments; Yuanbing Jiang and Hyebin Uhm set up the data-processing pipelines; Yuanbing Jiang, Hyebin Uhm, Fanny C. Ip, Li Ouyang, Albert Puig-Pijoan, Kin Y. Mok, John Hardy, Marc Suárez-Calvet, Henrik Zetterberg, Amy K. Y. Fu, and Nancy Y. Ip analyzed the data; and Yuanbing Jiang, Hyebin Uhm, Amy K. Y. Fu, and Nancy Y. Ip wrote the manuscript with input from all authors.

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CONFLICT OF INTEREST STATEMENT

Yuanbing Jiang, Fanny C. Ip, Amy K. Y. Fu, and Nancy Y. Ip are inventors of the related technology licensed to Cognitact. Yuanbing Jiang and Fanny C. Ip are co-founders, and Li Ouyang is a current staff member of Cognitact. John Hardy has served as a consultant for Eli Lilly and Eisai. Albert Puig-Pijoan has served on advisory boards for Schwabe Farma Ibérica. Marc Suárez-Calvet has served as a consultant and on advisory boards for Roche Diagnostics International Ltd. and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U. and Roche Farma, S.A. Henrik Zetterberg has served on scientific advisory boards and/or as a consultant for AbbVie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, NervGen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, AlzeCure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside of the submitted work). All other authors declare no conflicts of interest. Author disclosures are available in the Supporting Information S2.

CONSENT STATEMENT

All participants or legal guardians of participants with advanced dementia provided written informed consent for study participation and sample collection.

ROLE OF THE FUNDING SOURCE

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

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REFERENCES

1. Van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. 2023;388:9-21.
2. Mintun MA, Lo AC, Duggan Evans C, et al. Donanemab in early Alzheimer's disease. *N Engl J Med*. 2021;384:1691-1704.
3. Mummery CJ, Börjesson-Hanson A, Blackburn DJ, et al. Tau-targeting antisense oligonucleotide MAPTRx in mild Alzheimer's disease: a phase 1b, randomized, placebo-controlled trial. *Nat Med*. 2023;29:1437-1447.
4. Sims JR, Zimmer JA, Evans CD, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA*. 2023;330(6):512-527.
5. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol*. 2020;19:422-433.
6. Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA*. 2020;324:772-781.
7. Milà-Alomà M, Ashton NJ, Shekari M, et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease. *Nat Med*. 2022;28:1797-1801.
8. Quiroz YT, Zetterberg H, Reiman EM, et al. Plasma neurofilament light chain in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional and longitudinal cohort study. *Lancet Neurol*. 2020;19:513-521.
9. Preische O, Schultz SA, Apel A, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med*. 2019;25:277-283.
10. Candia J, Daya GN, Tanaka T, Ferrucci L, Walker KA. Assessment of variability in the plasma 7k SomaScan proteomics assay. *Sci Rep*. 2022;12:17147.
11. Davies MP, Sato T, Ashoor H, et al. Plasma protein biomarkers for early prediction of lung cancer. *eBioMedicine*. 2023;93:104686.
12. Jiang Y, Zhou X, Wong HY, et al. An IL1RL1 genetic variant lowers soluble ST2 levels and the risk effects of APOE-ε4 in female patients with Alzheimer's disease. *Nat Aging*. 2022;2:616-634.
13. Jiang Y, Zhou X, Ip FC, et al. Large-scale plasma proteomic profiling identifies a high-performance biomarker panel for Alzheimer's disease screening and staging. *Alzheimers Dement*. 2022;18:88-102.
14. Chatterjee P, Pedrini S, Stoops E, et al. Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease. *Transl Psychiatry*. 2021;11:27.
15. Whelan CD, Mattsson N, Nagle MW, et al. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. *Acta Neuropathol Commun*. 2019;7:1-14.
16. Sattlecker M, Kiddle SJ, Newhouse S, et al. Alzheimer's disease biomarker discovery using SOMAscan multiplexed protein technology. *Alzheimers Dement*. 2014;10:724-734.
17. Campo MD, Vermunt L, Peeters CF, et al. CSF proteome profiling reveals novel specific diagnostic biomarkers for dementia with Lewy bodies. *Alzheimers Dement*. 2022;18:e065449.
18. Bader JM, Geyer PE, Müller JB, et al. Proteome profiling in cerebrospinal fluid reveals novel biomarkers of Alzheimer's disease. *Mol Syst Biol*. 2020;16:e9356.
19. Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53:695-699.
20. American Psychiatric Association D, Association AP. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. American Psychiatric Association; 2013.
21. Lantero-Rodriguez J, Vrillon A, Fernández-Lebrero A, et al. Clinical performance and head-to-head comparison of CSF p-tau235 with p-tau181, p-tau217 and p-tau231 in two memory clinic cohorts. *Alzheimers Res Ther*. 2023;15:48.
22. Pangman VC, Sloan J, Guse L. An examination of psychometric properties of the mini-mental state examination and the standardized mini-mental state examination: implications for clinical practice. *Appl Nurs Res*. 2000;13:209-213.
23. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7:263-269.
24. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7:270-279.
25. Steyerberg EW, Eijkemans MJ, Harrell Jr FE, Habbema JDF. Prognostic modelling with logistic regression analysis: a comparison of selection and estimation methods in small data sets. *Stat Med*. 2000;19:1059-1079.
26. O'Bryant SE, Xiao G, Barber R, et al. A serum protein–based algorithm for the detection of Alzheimer disease. *Arch Neurol*. 2010;67:1077-1081.
27. Ray S, Britschgi M, Herbert C, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nat Med*. 2007;13:1359-1362.
28. Doecke JD, Laws SM, Faux NG. Blood-based protein biomarkers for diagnosis of Alzheimer disease. *Arch Neurol*. 2012;69:1318-1325.
29. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9:e95192.
30. Lehallier B, Gate D, Schaum N, et al. Undulating changes in human plasma proteome profiles across the lifespan. *Nat Med*. 2019;25:1843-1850.
31. Ganz P, Heidecker B, Hveem K, et al. Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. *JAMA*. 2016;315:2532-2541.
32. Mattsson N, Andreasson U, Zetterberg H, Blennow K, Initiative AsDN. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. *JAMA Neurol*. 2017;74:557-566.
33. Janelidze S, Stomrud E, Palmqvist S, et al. Plasma β-amyloid in Alzheimer's disease and vascular disease. *Sci Rep*. 2016;6:26801.
34. Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau-and amyloid-positron emission tomography. *Alzheimers Dement*. 2018;14:989-997.

35. Khan MJ, Desaire H, Lopez OL, Kamboh MI, Robinson RA. Why inclusion matters for Alzheimer's disease biomarker discovery in plasma. *J Alzheimers Dis*. 2021;79:1327-1344.
36. Zhang J, Dutta D, Köttgen A, et al. Plasma proteome analyses in individuals of European and African ancestry identify cis-pQTLs and models for proteome-wide association studies. *Nat Genet*. 2022;54:593-602.
37. Kim CX, Bailey KR, Klee GG, et al. Sex and ethnic differences in 47 candidate proteomic markers of cardiovascular disease: the Mayo Clinic proteomic markers of arteriosclerosis study. *PLoS One*. 2010;5:e9065.
38. Mao K, Zhang G. The role of PARP1 in neurodegenerative diseases and aging. *FEBS J*. 2022;289:2013-2024.
39. Chiu L-Y, Huang D-Y, Lin W-W. PARP-1 regulates inflammasome activity by poly-ADP-ribosylation of NLRP3 and interaction with TXNIP in primary macrophages. *Cell Mol Life Sci*. 2022;79:108.
40. Gu X, Dou M, Cao B, Jiang Z, Chen Y. Peripheral level of CD33 and Alzheimer's disease: a bidirectional two-sample Mendelian randomization study. *Transl Psychiatry*. 2022;12:427.
41. Griciuc A, Serrano-Pozo A, Parrado AR, et al. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron*. 2013;78:631-643.
42. Kishimoto. Cytokines and their receptors in cardiovascular diseases—role of gp130 signalling pathway in cardiac myocyte growth and maintenance. *Int J Exp Pathol*. 2000;81:1-16.
43. Santos GC, Silva DN, Fortuna V, et al. Leukemia inhibitory factor (LIF) overexpression increases the angiogenic potential of bone marrow mesenchymal stem/stromal cells. *Front Cell Dev Biol*. 2020;8:778.
44. Gupta VB, Hone E, Pedrini S, et al. Altered levels of blood proteins in Alzheimer's disease longitudinal study: results from Australian Imaging Biomarkers Lifestyle Study of Ageing cohort. *Alzheimers Dement*. 2017;8:60-72.
45. Perez-Frances M, van Gurp L, Abate MV, et al. Pancreatic Ppy-expressing γ-cells display mixed phenotypic traits and the adaptive plasticity to engage insulin production. *Nat Commun*. 2021;12:4458.
46. Asakawa A, Inui A, Yuzuriha H, et al. Characterization of the effects of pancreatic polypeptide in the regulation of energy balance. *Gastroenterology*. 2003;124:1325-1336.
47. Batterham R, Le Roux C, Cohen M, et al. Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab*. 2003;88:3989-3992.
48. Hansson O, Edelmayer RM, Boxer AL, et al. The Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease. *Alzheimers Dement*. 2022;18:2669-2686.
49. Ashton NJ, Janelidze S, Mattsson-Carlgren N, et al. Differential roles of Aβ42/40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring. *Nat Med*. 2022;28:2555-2562.
50. Mielke MM, Dage JL, Frank RD, et al. Performance of plasma phosphorylated tau 181 and 217 in the community. *Nat Med*. 2022;28:1398-1405.
51. Cummings J, Apostolova L, Rabinovici G, et al. Lecanemab: appropriate use recommendations. *J Prev Alzheimers Dis*. 2023;10:362-377.
52. Bradford A, Kunik ME, Schulz P, Williams SP, Singh H. Missed and delayed diagnosis of dementia in primary care: prevalence and contributing factors. *Alzheimer Dis Assoc Disord*. 2009;23:306.
53. Lang L, Clifford A, Wei L, et al. Prevalence and determinants of undetected dementia in the community: a systematic literature review and a meta-analysis. *BMJ Open*. 2017;7:e011146.
54. Jonsson T, Stefansson H, Steinberg S, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med*. 2013;368:107-116.
55. Heneka MT, Kummer MP, Stutz A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*. 2013;493:674-678.
56. Kellar D, Craft S. Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. *Lancet Neurol*. 2020;19:758-766.
57. Montagne A, Nation DA, Sagare AP, et al. APOE4 leads to blood–brain barrier dysfunction predicting cognitive decline. *Nature*. 2020;581:71-76.
58. Heidari F, Ansstas G, Ajamian F. CD33 mRNA has elevated expression levels in the leukocytes of peripheral blood in patients with late-onset Alzheimer's disease. *Gerontology*. 2022;68:421-430.
59. Griciuc A, Federico AN, Natasan J, et al. Gene therapy for Alzheimer's disease targeting CD33 reduces amyloid beta accumulation and neuroinflammation. *Hum Mol Genet*. 2020;29:2920-2935.
60. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. *JAMA*. 1997;278:1349-1356.
61. Zhou X, Chen Y, Mok KY, et al. Non-coding variability at the APOE locus contributes to the Alzheimer's risk. *Nat Commun*. 2019;10:3310.
62. Matthews KA, Xu W, Gaglioti AH, et al. Racial and ethnic estimates of Alzheimer's disease and related dementias in the United States (2015-2060) in adults aged≥ 65 years. *Alzheimers Dement*. 2019;15:17-24.
63. Morris JC, Schindler SE, McCue LM, et al. Assessment of racial disparities in biomarkers for Alzheimer disease. *JAMA Neurol*. 2019;76:264-273.
64. Schindler SE, Karikari TK, Ashton NJ, et al. Effect of race on prediction of brain amyloidosis by plasma Aβ42/Aβ40, phosphorylated tau, and neurofilament light. *Neurology*. 2022;99:e245-e257.
65. O'Bryant S, Petersen M, Hall J, et al. Characterizing plasma NfL in a community-dwelling multi-ethnic cohort: results from the HABLE study. *Alzheimers Dement*. 2022;18:240-250.
66. Ferreira D, Nordberg A, Westman E. Biological subtypes of Alzheimer disease: a systematic review and meta-analysis. *Neurology*. 2020;94:436-648.
67. Vogel JW, Young AL, Oxtoby NP, et al. Four distinct trajectories of tau deposition identified in Alzheimer's disease. *Nat Med*. 2021;27:871-881.
68. Ossenkoppele R, Lyoo CH, Sudre CH, et al. Distinct tau PET patterns in atrophy-defined subtypes of Alzheimer's disease. *Alzheimers Dement*. 2020;16:335-344.
69. Neff RA, Wang M, Vatansever S, et al. Molecular subtyping of Alzheimer's disease using RNA sequencing data reveals novel mechanisms and targets. *Sci Adv*. 2021;7:eabb5398.

SUPPORTING INFORMATION

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

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