

Background

Amnestic mild cognitive impairment (aMCI) is widely regarded as a prodromal stage of Alzheimer's disease (AD), yet its subtle clinical presentation often leads to underdiagnosis in routine neurological practice. Although advances in cerebrospinal fluid biomarkers, positron emission tomography, and plasma-based markers, such as p-tau217, have improved early AD detection, their clinical application remains limited by high costs, invasiveness, and restricted accessibility. Consequently, there is a pressing need for scalable, non-invasive, and cost-effective plasma biomarkers for early aMCI screening. Recent advances in proteomics, particularly liquid chromatography–tandem mass spectrometry (LC-MS/MS), have enabled comprehensive profiling of plasma proteins. However, the wide dynamic range of plasma protein abundance hampers the detection of low-abundance proteins. Deep plasma proteomics using functional bio-magnetic bead enrichment offers a promising strategy to overcome this limitation and facilitate the discovery of novel plasma biomarkers for early aMCI detection.

Methods

Eighty-four participants (40 aMCI and 44 cognitively normal [CN]) were recruited from the Shenzhen Multi-Modal Aging Research (STAR) cohort. Participants were matched for sex, family history, smoking, and drinking status, while CN participants were slightly younger and had higher education than aMCI participants. Low-abundance plasma proteins were detected using deep proteomics with biofunctional magnetic bead enrichment. An early diagnostic model for aMCI was developed using bioinformatics and machine learning and compared with plasma biomarkers measured by the Simoa assay, including p-tau217, p-tau181, A β ₁₋₄₀, and A β ₁₋₄₂.

Table 1. Demographic data and neuropsychological scores for all participants

	CN(n=44)	aMCI(n=40)	<i>p</i>
Age, years (Mean $\pm$ SD)	59.55 $\pm$ 4.18	65.25 $\pm$ 6.49	<0.001
Gender (male/female)	13/31	11/29	0.838
Education, years (Mean $\pm$ SD)	13.55 $\pm$ 3.40	9.95 $\pm$ 3.57	<0.001
Family history (yes/no)	7/26	5/20	0.912
Smoking (yes/no)	1/38	5/26	0.045
Drinking(yes/no)	8/31	5/27	0.620
MMSE (Mean $\pm$ SD)	29.00 $\pm$ 0.96	26.80 $\pm$ 1.54	<0.001
MoCA (Mean $\pm$ SD)	27.20 $\pm$ 1.19	19.20 $\pm$ 2.46	<0.001
ADL (Mean $\pm$ SD)	20.16 $\pm$ 0.37	20.45 $\pm$ 1.06	0.091

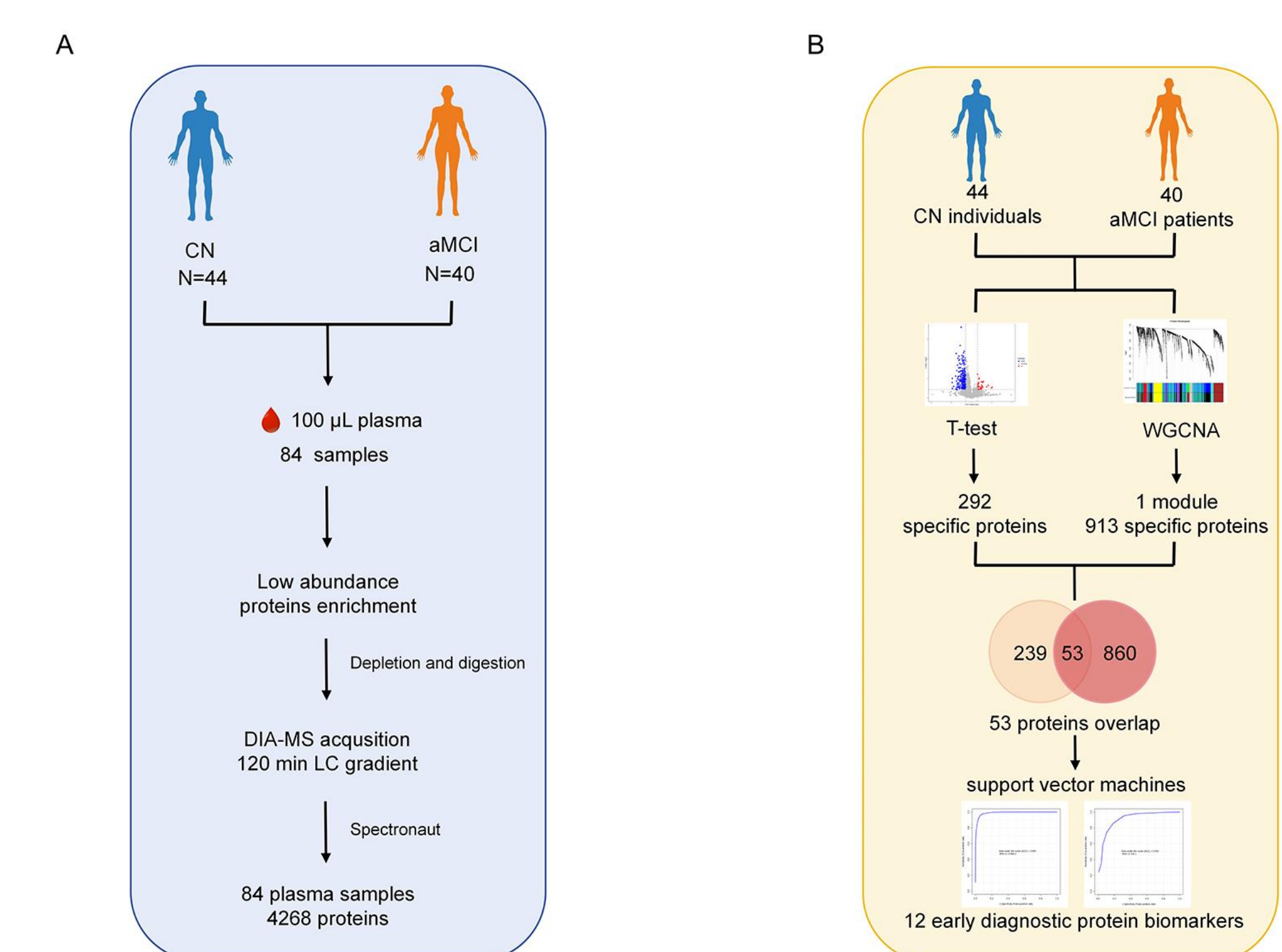


Fig. 1 Schematic of the workflow for proteomics analyses and machine learning. (A) Workflow based on plasma deep proteomics. (B) Exploring the workflow of early diagnostic biomarkers of aMCI.

Results

A total of 4,268 plasma proteins were identified, among which 292 were differentially expressed between CN and aMCI groups. Integration of differential expression analysis and WGCNA highlighted 53 candidate proteins, from which machine learning selected an optimal 12-protein panel. This panel achieved excellent diagnostic performance (AUC = 0.992 in the training set and 0.933 in the testing set), and targeted proteomics confirmed consistent expression trends for most proteins. Compared with the 12-protein panel (AUC = 0.933; accuracy = 93.9%), plasma biomarkers measured by the Simoa assay showed lower diagnostic performance (AUC = 0.884; accuracy = 24.4%).

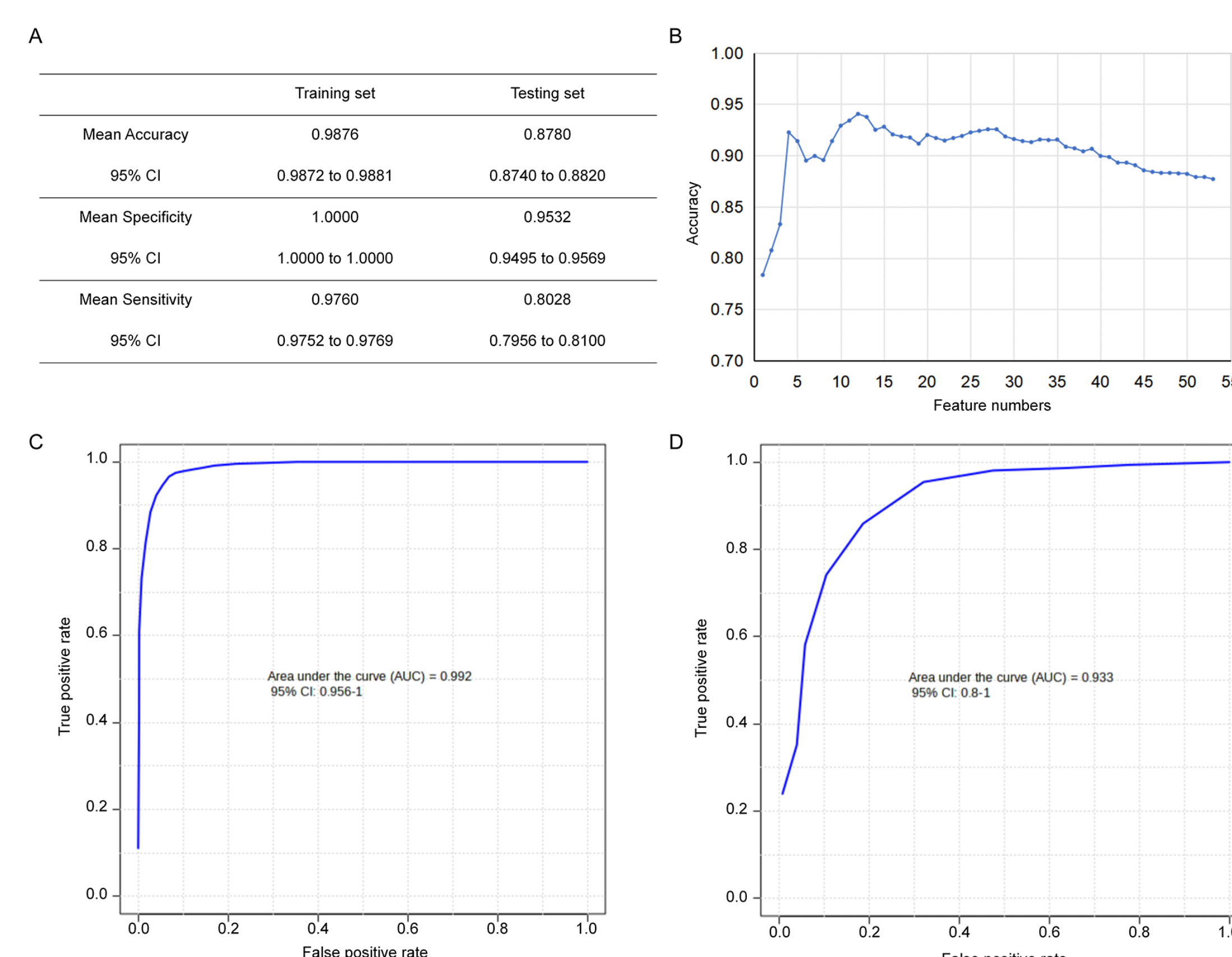


Fig. 2 The 12-protein biomarker assay accurately classifies the early stage of aMCI. (A) Statistics of the SVM model classification efficacy. (B) Feature screening based on the SVM model. (C-D) Receiver operating characteristic (ROC) curves showing the performance of the 12-protein biomarker panel in the training set and testing set.

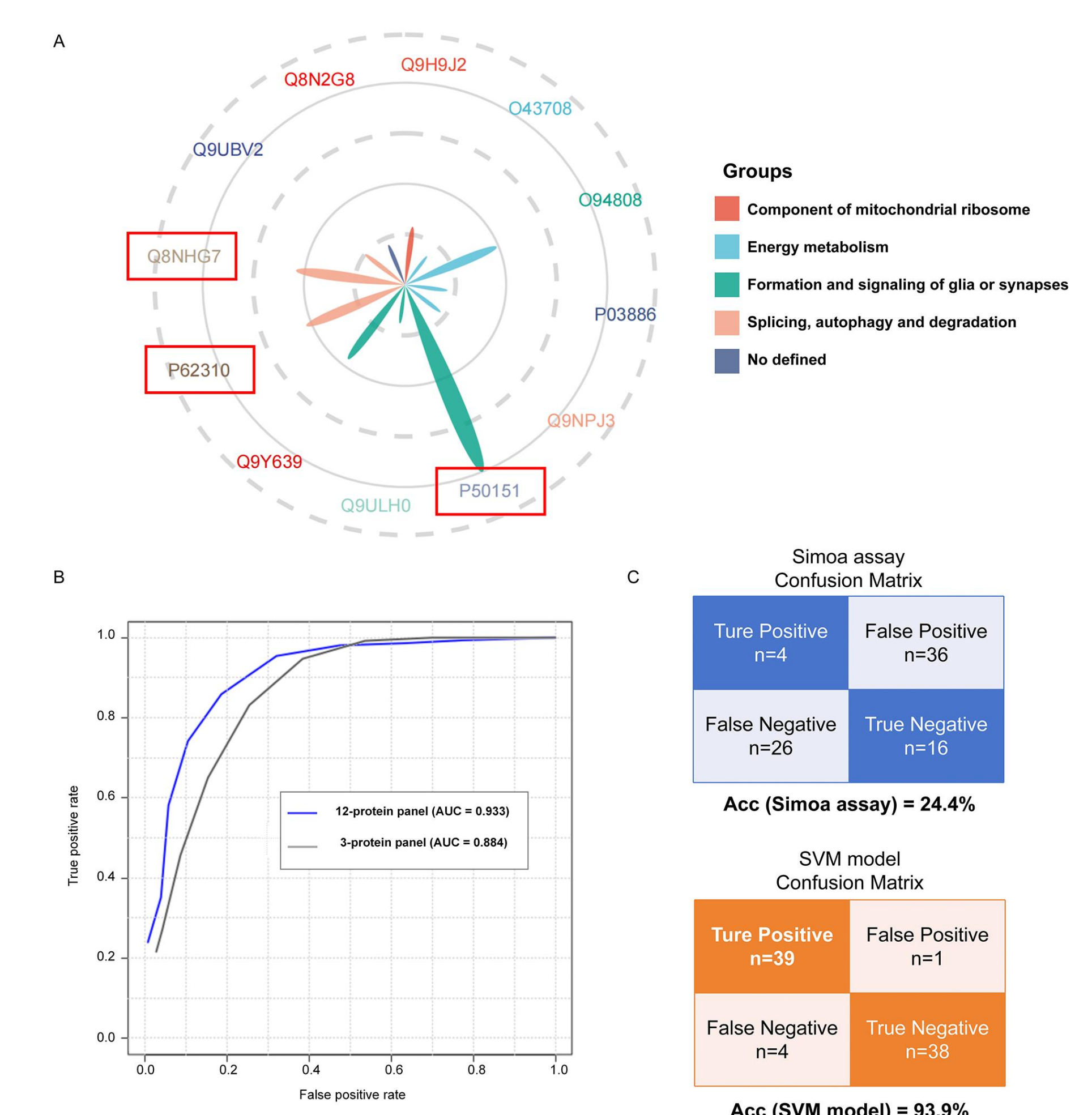


Fig. 3 Comparison of the 12-protein panel and Simoa Assay. (A) Weights and biological functions of individual proteins in the 12-protein panel. The weight of the center is 0; the farther away from the center, the greater the weight, indicating that the protein is more important in the panel. Different colors represent different biological functions, as detailed in the legend. (B) Receiver operating characteristic (ROC) curves showing the performance of the 12-protein panel and 3-protein panel in the testing set. 95% CI of the 12-protein panel is 0.8-1, and 95% CI of the 3-protein panel is 0.75-1. (C) Confusion matrix and Accuracy for the Simoa assay and SVM model.

Conclusion

The deep plasma proteomics–derived 12-protein panel provides a cost-effective method for early aMCI detection, potentially improving screening efficacy in clinical practice.