Received: 30 January 2020 | Accepted: 27 February 2020 | Published online: 8 May 2020

DOI: 10.1002/trc2.12018

Alzheimer's & Dementia
Translational Research & Clinical Interventions

RESEARCH ARTICLE

Plasma proteomic signatures predict dementia and cognitive impairment

Toshiko Tanaka[1] | **Robert Lavery**[1] | **Vijay Varma**[2] | **Giovanna Fantoni**[3] | **Marco Colpo**[4] | **Madhav Thambisetty**[2] | **Julian Candia**[5] | **Susan M. Resnick**[6] | **David A. Bennett**[7] | **Angelique Biancotto**[8] | **Stefania Bandinelli**[4] | **Luigi Ferrucci**[1]

[1]Translational Gerontology Branch, National Institute on Aging, NIH, Baltimore, Maryland

[2]Laboratory of Behavioral Neuroscience, National Institute on Aging, NIH, Baltimore, Maryland

[3]National Institute on Aging (NIA), Intramural Research Program (IRP), Clinical Research Core (CRC)

[4]Geriatric Unit, Azienda Sanitaria di Firenze, Florence, Italy

[5]Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland

[6]Laboratory of Behavioral Neuroscience, National Institute of Aging, Baltimore, Maryland

[7]Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, Illinois

[8]Precision Immunology, Immunology and Inflammation Research Therapeutic Area, Sanofi, Cambridge, MA, USA

Correspondence
Toshiko Tanaka, Translational Gerontology Branch, National Institute on Aging, NIH, Baltimore, MD 21224.
Email: tanakato@mail.nih.gov

Funding information
Italian Ministry of Health; U.S. National Institute on Aging, Grant/Award Numbers: 263 MD 9164, 263 MD 821336; U.S. National Institute on Aging, Grant/Award Numbers: N.1-AG-1-1, N.1-AG-1-2111; U.S. National Institute on Aging, Grant/Award Number: N01-AG-5-0002; Intramural Research Program of the National Institute on Aging, National Institutes of Health, Baltimore, Maryland; NIA, Grant/Award Numbers: P30AG10161, R01AG15819

Abstract

Introduction: Biomarker discovery of dementia and cognitive impairment is important to gather insight into mechanisms underlying the pathogenesis of these conditions.

Methods: In 997 adults from the InCHIANTI study, we assessed the association of 1301 plasma proteins with dementia and cognitive impairment. Validation was conducted in two Alzheimer's disease (AD) case-control studies as well as endophenotypes of AD including cognitive decline, brain amyloid burden, and brain volume.

Results: We identified four risk proteins that were significantly associated with increased odds (peptidase inhibitor 3 (PI3), trefoil factor 3 (TFF3), pregnancy associated plasma protein A (PAPPA), agouti-related peptide (AGRP)) and two protective proteins (myostatin (MSTN), integrin aVb5 (ITGAV/ITGB5)) with decreased odds of baseline cognitive impairment or dementia. Of these, four proteins (MSTN, PI3, TFF3, PAPPA) were associated cognitive decline in subjects that were cognitively normal at baseline. ITGAV/ITGB5 was associated with lower brain amyloid burden, MSTN and ITGAV/ITGB5 were associated with larger brain volume and slower brain atrophy, and PI3, PAPPA, and AGRP were associated with smaller brain volume and/or faster brain atrophy.

Discussion: These proteins may be useful as non-invasive biomarkers of dementia and cognitive impairment.

KEYWORDS

brain imaging, cognitive impairment, cognitive trajectories, dementia, proteomics

1 | INTRODUCTION

Dementia and cognitive impairment are significant contributors to disability and loss of independence. As of 2001, the Alzheimer's Disease International panel estimated that 24.3 million individuals were affected by dementia worldwide, and projections indicate that up to 115.4 million people will be affected by 2055.[1] Currently, there are no known effective therapies for the prevention or treatment of dementia. Identifying biomarkers of dementia and cognitive impairment that precedes dementia can provide insight into the mechanisms of disease progression and uncover potential targets for the development of effective treatments. Blood biomarkers offer the advantage of high-throughput, repeated measurements, and low cost.[2,3] Furthermore, biomarkers could serve as a useful tool in identifying those at risk for developing dementia at a time when disease progression is most likely to be responsive to treatments.

Discovery proteomics allows a large-scale characterization and quantification of proteins in target biological specimens. Protein abundances can change throughout the disease process and thus can be used as a biomarkers for diagnosis, to monitor the progression of disease pathology and to track the effects of therapeutic interventions.[4] Although the study of biomarkers is most useful in biological fluids, such as serum and plasma, performing proteomics is particularly challenging in these samples because of the presence of highly abundant proteins, such as albumin and transferrin, that mask the measurement of lower abundant proteins, which may be most informative.[5] In addition, the extremely wide dynamic range of proteins in the blood limits the use of standard mass spectrometry methods for capturing the whole circulating proteome.[5] Alternative highly sensitive methods have been developed that allow the quantification of a specific subset of proteins in biological fluids, including the SOMAscan technology that uses highly specific aptamers. Several studies have correlated circulating protein biomarkers with Alzheimer's disease (AD) and cognitive function, but findings of these studies have been heterogeneous.[2] Using the SOMAscan technology, the AddNeuroMed study identified α1-antichymotrypsin, pancreatic prohormone (PPY), trypsin, and calcium/calmodulin dependent protein kinase II alpha (CAMK2A) as candidate protein biomarkers of AD.[6,7] Other studies have examined AD endophenotypes including cognitive decline, brain atrophy, and rate of cognitive decline in AD patients.[8,9] These studies have focused primarily on biomarkers of disease progression. Exploring proteins associated with clinical diagnosis of dementia as well as sensitive endophenotypes among participants prior to the onset of dementia and cognitive impairment may be more effective in identifying proteins biomarkers of disease.

In our present study, we took a staged approach to identifying blood-based biomarkers cross-sectionally associated with dementia and cognitive impairment (supplementary Figure S1). In the discovery step, we used data from the Invecchiare in Chianti (InCHIANTI) study and tested the associations of plasma proteins with dementia and cognitive impairment to identify a set of candidate dementia or cognitive impairment associated proteins. In the second phase, we validated the candidate proteins with various dementia endophenotypes including longitudinal cognitive change in InCHIANTI, discrimination between AD and control in the Baltimore Longitudinal Study of Aging (BLSA) and the Religious orders Study (ROS), and associations with amyloid burden and brain volume/rates of atrophy in BLSA.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the prior cross-sectional and longitudinal epidemiological studies from the international literature for studies that conducted proteomic biomarker discovery of dementia, cognitive impairment, and Alzheimer's disease. This review was conducted through PubMed using the following search keyword terms: dementia OR Alzheimer's disease OR cognitive function OR proteomics OR aptamer OR biomarkers.
2. Interpretation: In our primary analysis, we identify six proteins that are associated with dementia and cognitive impairment. We find that four of these proteins are predictive of cognitive decline over a 15-year follow-up period in subjects who are free of cognitive impairment at baseline. In two independent studies, we show that each protein is associated with at least one endophenotype of dementia: brain amyloid burden, brain volume, and brain atrophy. These findings together provide strong evidence that these six proteins are strong candidate proteomic biomarkers of dementia and cognitive impairment and may be useful in identifying at-risk individuals.
3. Future directions: This manuscript identifies six potential biomarkers of dementia and cognitive impairment. Future studies should include validating the finding in longitudinal studies with data on incident dementia or cognitive impairment to test whether these proteins are predictive of the development of dementia or cognitive impairment. These studies will be crucial in evaluating the utility of these proteins as biomarkers that could be used clinically or in intervention studies to identify at-risk populations.

2 | METHODS

2.1 | Study participants and design

The Invecchiare in Chianti (or InCHIANTI) study is a population-based prospective cohort study conducted in the Chianti region in Tuscany, Italy and aimed at identifying factors that influence loss of mobility with aging. Details of the study have been described[10]; briefly, 1453 individuals from 20 to 102 years of age were randomly selected based on city registries. Overnight fasted blood and plasma samples were

stored for genomic DNA extraction, and measurement of plasma proteins. Of the 1453 InCHIANTI participants, 997 who had valid baseline plasma proteomic and cognitive measurements were included in this study. The primary proteomic analysis examined the association of individual protein abundances with prevalent dementia and cognitive impairment at baseline (Supplementary Figure S1). The study protocol was approved by the Italian National Institute of Research and Care of Aging Institutional Review Board and the Medstar Research Institute (Baltimore, MD).

Sociodemographic information (age, sex, and years of education) was obtained during the structured interview. The number of co-morbidities were defined based on 15 chronic conditions as previously defined using standard criteria that combined information from self-reported medical history, medication use, medical documents, and a clinical medical examination.[11] The 14 diseases considered included hypertension, diabetes, ischemic heart disease, congestive heart failure, stroke, chronic obstructive pulmonary disease, cancer, Parkinson's disease, hip fracture, and lower extremity joint disease, anemia, chronic kidney disease, and peripheral arterial disease.

To validate primary findings from the InCHIANTI study, we used serum samples from two additional cohort studies (supplementary Figure S1). The Baltimore Longitudinal Study of Aging (or BLSA) is a prospective cohort study administered by the National Institute on Aging (NIA).[12] Clinical evaluations, including radiological, neurological, and laboratory evaluations, are conducted every 2 years; participants older than 80 years received annual assessments starting in 2003. BLSA participants (n = 154) considered in this report included individuals from an age-matched case-control study of AD (ie, converter/non-converter) described previously.[13] Briefly, participants defined as "converters" were cognitively normal at the initial blood draw and developed incident AD based on consensus clinical diagnosis during follow-up ≈5 years later. These subjects were age- and sex-matched to non-converters defined as participants who were cognitively normal at baseline and who remained cognitively normal over a similar follow-up interval. From this analysis, we used the serum proteomic measurement at dementia onset to match the cross-sectional analysis performed in the InCHIANTI study. In addition, brain amyloid burden, brain volumes, and rates of atrophy were measured in a subset of BLSA participants (n = 146) in the longitudinal neuroimaging substudy (BLSA-NI)[14]; similar to the InCHIANTI study design for exploring longitudinal trajectories of cognitive decline, all BLSA participants in the neuroimaging study were cognitively normal at baseline.

The Religious Orders Study (ROS) is a prospective cohort study of older catholic nuns, priests, and monks.[15] All participants enroll without known dementia and agree to annual detailed clinical evaluation and organ donation at death. A subset agreed to annual blood donation. The study was approved by an Rush University Medical Center Institutional Review Board. All participants provided signed an informed consent, an Anatomic Gift Act, and a repository consent to allow their biospecimens and data to be shared. ROS participants (n = 42) included in this study provided serum. The same criteria used in BLSA to define converters and non-converters were used in ROS.

2.2 | Blood proteomic assays

Proteomic profiles for 1322 SOMAmers in plasma (InCHIANTI) and serum (BLSA, ROS) were assessed using the 1.3K SOMAscan assay at the Trans-NIH Center for Human Immunology and Autoimmunity, and Inflammation (CHI), National Institute of Allergy and Infectious Disease, National Institutes of Health (Bethesda, MD, USA). SOMAscan assay platform includes 1322 SOMAmer Reagents, and of these 12 are hybridization controls, 4 four are viral proteins (human papilloma virus [HPV] type 16, HPV type 18, isolate BEN, isolate LW123), and 5 are nonspecifically targeted SOMAmers (P05186; ALPL, P09871; C1S, Q14126;DSG2, Q93038; TNFRSF25, Q9NQC3; RTN4). Thus, the final analysis was conducted in 1301 SOMAmer Reagents that target 1297 unique proteins. Finally, the protein panel includes four proteins that are rat homologues (P05413; FABP3, P48788; TINNI2, P19429; TINNI3, P01160; NPPA) of human proteins. The experimental process utilized in proteomic assessment and normalization has been reported previously.[16] Protein concentrations were reported as relative abundance of SOMAmer reagents. The data readout is relative fluorescence units (RFUs) and is directly proportional to the reported relative abundance of SOMAmer reagents. The data normalization process includes hybridization, control normalization, median signal normalization, and calibration normalization, as detailed previously.[16]

2.3 | Neurocognitive assessment

2.3.1 | InCHIANTI study

In the InCHIANTI study, cognitive function was assessed using a two-stage screening procedure as described previously (supplementary Figure S2).[17] Briefly, global cognitive function was assessed using the Mini-Mental State Examination (MMSE) score that was adjusted for education at baseline (1998 to 2000) and four follow-up visits at 2001 to 2003, 2004 to 2006, 2007 to 2009, and 2013 to 2014.[18] Participants with baseline MMSE score >26 were considered free of dementia, whereas those with a score ≤21 were considered possibly cognitively impaired and directly scheduled for the second-stage screening procedure. Participants with an MMSE score between 22 and 26 (N = 539) received additional neuropsychological tests assessing memory (paired-words test), concentration/attention (digit test from the Weschler Adult Intelligence Scale), and visuo-spatial ability (the Caltagirone drawings).[19] The second-stage screening was performed by geriatricians and a psychologist with longstanding clinical experience in the evaluation of older patients with cognitive impairment. A diagnosis of "dementia syndrome" independent of the etiology was established using a standard evaluation protocol based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria.

At baseline, participants were categorized into three groups (Supplementary Figure S2): (1) participants with normal cognitive function (ie, MMSE score >23, no diagnosis of dementia, and no activities of daily living (ADL)/instrumental ADL (IADL) disability attributable to cognitive impairment) (n = 883); (2) participants with cognitive impairment but not dementia (ie, those with an MMSE score <23 and/or any degree of ADL/IADL disability attributable to cognitive impairment) (n = 86); and (3) participants diagnosed with dementia (n = 28).

2.3.2 | Baltimore Longitudinal Study on Aging and Religious Orders Study

Procedures for determining cognitive status in the BLSA[20] and ROS[21] have been described in detail previously. Briefly, in the BLSA study, cognitive status was determined at consensus diagnostic conferences in participants who obtained at Clinical Dementia Rating Score of 0.5 or greater or made four or more errors on the Blessed Information Memory Concentration Test. Longitudinal participant data reviewed during case conferences included neuropsychological assessments, medication history, self-reported diagnoses of comorbid medical conditions, MRI radiologic interpretations, and laboratory evaluation for reversible causes of cognitive impairment (eg, serum thyroid-stimulating hormone [TSH] and vitamin B_{12} levels). Dementia diagnosis was based on the Diagnostic and Statistical Manual (DSM)-III-R criteria.[22] AD diagnosis was based on the National Institute of Neurological and Communication Disorders and Stroke—Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[23]

In ROS, cognitive status was determined based on a three-stage process including computer scoring of cognitive tests, clinical judgment of a neuropsychologist, and diagnostic classification by a clinician.[24,25] Follow-up diagnoses were performed blinded to all prior data.

2.4 | *APOE* genotyping

In the InCHIANTI study, apolipoprotein E (*APOE*) variant genotypes (e2, e3, e4) was defined by two single nucleotide polymorphisms (SNPs) rs429358 and rs7412. Genotyping of these two SNPs were conducted using TaqMan assays (Applied Biosystems, Inc. [ABI], Foster City, CA) following manufacturer's instructions. For analysis, subjects were grouped into $\varepsilon 4$ carrier versus non-$\varepsilon 4$ carriers.

2.5 | Statistical analysis

Of the 1453 InCHIANTI subjects, 997 subjects had complete proteomic and cognitive data. Comparison of baseline characteristics was conducted using analysis of variance for continuous variables and chi-square test for categorical variables. For proteomic analysis, protein RFU values were natural-log transformed and outliers outside of three standard deviations (SD) were removed, and then protein values were standardized. We conducted analyses on the protein z-scores for both cross-sectional and longitudinal change of cognitive status.

For the cross-sectional analysis at baseline, we tested the associations of protein abundances with cognitive status (dementia, cognitive impairment, cognitively normal) using logistic regression. The comparisons that were carried out included (1) dementia versus normal, (2) cognitively impaired versus normal, and (3) dementia or cognitively impaired versus normal. A final analysis that considered the three categories as an ordinal outcome (dementia, cognitively impaired, cognitively normal) was carried out using ordinal logistic regression. For proteins that were associated with cognitive status at baseline in any of the analyses described, we tested whether they were associated with differential rate of change in MMSE score over the follow-up. For these subjects, incident dementia at follow-up was not ascertained. First, trajectories of MMSE over the follow-up period were modeled using linear mixed-effects models, using time as a random effect. Because there was little variability in MMSE at baseline, models with fixed intercept with random slope were implemented. To assess associations of baseline protein values with trajectories of MMSE, we tested for significance of the interaction term between protein abundances and time. For all analyses, models were adjusted for age in years, sex, study site, years of education, *APOE* $\varepsilon 4$ carrier status, and number of co-morbidities. For each protein-wide analyses, an false discovery rate (FDR) (Benjamini-Hochberg) adjusted q-value of <.05 was considered as significant.

2.6 | Validation—differences in candidate proteins by cognitive status

To validate target proteins identified in the index InCHIANTI study, we performed cross-sectional analyses similar to the one performed in the InCHIANTI study by exploring protein differences in AD and control groups at the time of symptom onset in the BLSA (AD = 74, control = 67) and ROS (AD = 25, control = 42) case-control data. Logistic regression models were used to test for differences between groups; the predictor of interest was protein concentration (natural log transformed and outliers ± 3 SD excluded) and covariates included centered age, race, and sex. Positive and negative coefficients indicated that higher protein concentration was associated with higher and lower, respectively, log odds of being converter compared to a non-converter. For validation analysis, a *P*-value of <.05 was considered as significant.

2.7 | Association of proteins with brain atrophy and amyloid status

To validate target proteins identified in the index InCHIANTI study, we additionally explored associations between proteins and endophenotypes of AD including brain amyloid burden and brain atrophy in the BLSA neuroimaging cohort (N = 135). BLSA participants in the neuroimaging study underwent serial brain scans with an average follow-up period of 2.48 years (range: 0 to 8.16 years). Brain volume was

measured using a 3T Philips Achieva Magnetic Resonance Imaging (MRI) system to acquire magnetization-prepared rapid gradient echo (MPRAGE) scans (repetition time = 6.8 ms, echo time = 3.2 ms, flip angle = 8°, image matrix = 256 × 256, 170 slices, pixel size = 1 × 1 mm, slice thickness = 1.2 mm; sagittal acquisition). Anatomical labels and global and regional brain volumes were obtained using Multi-atlas region Segmentation using Ensembles of registration algorithms and parameters (MUSE).[26] For longitudinal analyses, we selected a set of brain regions based on prior work suggesting that those regions are sensitive to age-related change[27]; these included total brain volume, total ventricular volume, total gray matter, frontal gray matter, temporal gray matter, parietal gray matter, occipital gray matter, total white matter, frontal white matter, temporal white matter, parietal white matter, occipital white matter, hippocampus, entorhinal cortex, amygdala, para-hippocampal gyrus, fusiform gyrus, and precuneus. BLSA participants additionally underwent 11C-PiB PET scans to assess brain amyloid β burden. A detailed description of acquisition and preprocessing procedures has been published previously.[28] Individuals were characterized as PiB+ or PiB− based on a mean cortical distribution volume ratio (DVR) threshold of 1.066, based on a two-class Gaussian mixture model.[28]

We first explored differences in baseline serum protein abundances in the PiB+ group compared to the PiB− group using logistic regression models; the predictor of interest was serum protein concentration (natural log transformed and outliers ± 3 SD excluded) and covariates included centered age, race, and sex. Positive and negative coefficients indicated that higher protein concentration was associated with higher and lower log odds, respectively, of being PiB+ compared to PiB−.

We then explored associations between serum protein concentrations and mean cortical DVR, a measure of global amyloid burden, as well as precuneus DVR using linear regression in individuals who were PiB+[28]; the predictor of interest was protein concentration (natural log transformed and outliers ± 3 SD excluded) and covariates included centered age and sex (race was excluded from analyses due to smaller sample size). Positive and negative coefficients indicated that higher serum protein concentration was associated with a higher and lower brain amyloid accumulation, respectively.

Finally, we explored associations between protein concentrations (measured at baseline defined as the earliest time point where both protein and imaging data was available prior to longitudinal brain imaging data) and rates of change in brain atrophy using linear mixed-effects models with a random intercept term. The outcome of interest was the brain volume measure and the predictors of interest were serum protein concentration (indicating the baseline, cross-sectional effect) and the interaction between protein concentration and time (indicating the longitudinal effect). We additionally included mean-centered baseline age (at blood draw), sex, race, intracranial volume (ICV), and time in days between baseline and follow-up visits (baseline indicate as time = 0) and the two-way interaction of each predictor with time. Positive and negative coefficients for baseline, cross-sectional effects indicated that higher protein concentration was associated with larger and smaller brain volumes, respectively; and for longitudinal effects indicated that higher protein concentration was associated with slower and faster rates, respectively, of brain atrophy (other than for ventricular volume were positive and negative coefficients indicated faster and slower rates, respectively).

3 | RESULTS

3.1 | Baseline proteomic analysis of dementia, cognitive impairment, and cognitive function

Of the 1453 participants enrolled in the InCHIANTI study, 997 participants with proteomic and cognitive measurements at baseline were used in the analysis (supplementary Table S1). Compared to subjects with complete data, those without proteomic data (N = 456) were older, were more likely to live in Bagno a Ripoli, had more years of education, had fewer co-morbidities, and had lower MMSE scores ($P < .05$). Among the 997 subjects with complete data, 8.6% (N = 86) had cognitive impairment without dementia and 2.8% (N = 28) had dementia at baseline (Table 1). Participants with cognitive impairment and dementia at baseline had higher mean age, fewer years of education, and lower MMSE scores compared to participants with normal cognitive function ($P < .05$).

We then tested the associations between protein abundances with cognitive status at baseline in the whole study population. In the initial analysis, protein abundances in subjects with dementia or cognitive impairment were compared with subjects that were cognitively normal independently in two separate models. There were no proteins associated with either dementia or cognitive impairment. To increase power, a second analysis was conducted where subjects with dementia and cognitive impairment were combined and compared to cognitively normal subjects. There were three proteins associated with combined cognitive impairment and dementia (supplementary Table S2; Figure 1A through C). For two proteins, myostatin (MSTN; $OR_{SD:}$ 0.58 [0.45 to 0.75], q = 0.012 and integrin aVb5 (ITGAV/ITGB5; OR_{SD}: 0.57 [0.44 to 0.74], q = 0.012), higher serum concentration was associated with a lower odds of having cognitive impairment or dementia (supplementary Table S2). One protein, peptidase inhibitor 3 (PI3; odds ratio per standard deviation (OR_{SD}): 1.8 [1.4 to 2.3], q = 0.006) was associated with a higher odds of having cognitive impairment or dementia. In the final analysis, we used ordinal logistic regression to compare the associations between the three cognitive groups assuming proportional odds from cognitively normal, cognitively impaired, and dementia. There were six proteins that were associated with cognitive status (Table 2; Figure 1A through F). For four proteins, PI3 (OR_{SD}: 1.78 [1.40 to 2.27], q = 0.004), trefoil factor 3 (TFF3; OR_{SD}: 1.72 [1.32 to 2.23], q = 0.015), pregnancy associated plasma protein A (PAPPA; OR_{SD}: 1.68 [1.30 to 2.18], q = 0.019), and agouti-related peptide (AGRP; OR_{SD}: 1.55 [1.23 to 1.94], $P = .035$), higher concentration was associated with a higher odds of being in the higher cognitive impairment group. For MSTN ($OR_{SD:}$ 0.57 [0.44 to 0.73], $P = .004$) and ITGAV/ITGB5 (OR_{SD}: 0.58 [0.46 to 0.74], q = 0.007), higher abundance was associated with a lower odds of being in the higher cognitive impairment group. In 883 subjects who were cognitively normal at baseline, none of the proteins

TABLE 1 Characteristics of InCHIANTI participants by cognitive status at baseline

	Normal	Cognitively impaired	Dementia	*P*
n	883	86	28	
Age (years)	64.6 ± 15.3	78.5 ± 8.4	81.6 ± 7.4	<.001
Sex (% women)	587 (52.7%)	174 (71.3%)	50.0 (60.9%)	.053
Location-Bagno a Ripoli (%)	581 (52.5%)	105 (47.8%)	39.0 (47.8%)	.073
Education (years)	7.2 ± 4.4	4.1 ± 2.5	2.9 ± 2.1	<.001
Number of co-morbidities	1.0 ± 1.1	1.9 ± 1.5	2.4 ± 1.7	<.001
MMSE	26.9 ± 2.5	21.8 ± 2.5	13.5 ± 6.8	<.001
APOE ε4 carrier status	175 (16.36%)	32.0 (16.08%)	15.0 (24.19%)	.084

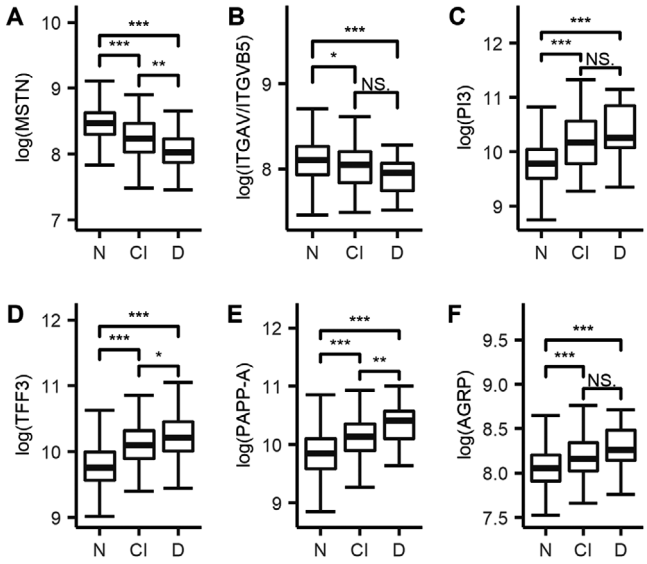

FIGURE 1 Protein abundances of eight plasma proteins associated with cognitive function at baseline. Box-plots representing mean and standard error of the logged relative abundances of (A) myostatin (MSTN), (B) integrin aVb5 (ITGAV/ITGVB), (C) peptidase inhibitor 3 (PI3), (D) trefoil factor 3 (TFF3), (E) pregnancy associated plasma protein A (PAPPA), and (F) agouti-related peptide (AGRP) in individuals with normal cognitive function (N), cognitive impairment (CI), and dementia (D). Differences between groups were calculated with adjustment for age, sex, study site, years of education, *APOE* ε4 carrier status, and number of co-morbidities. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

examined were significantly associated with MMSE at the baseline visit (supplementary Table S2).

3.2 | Proteomic analysis of cognitive decline

To test whether the candidate proteins cross-sectionally associated with dementia and cognitive impairment can be an early marker of disease, we investigated their association with cognitive decline in subjects who were cognitively normal at baseline (n = 883). We assessed the association of baseline protein abundances with trajectories of MMSE decline over a 15-year follow-up in subjects who were cognitively normal at baseline. The average decline in MMSE was 0.25 units/y. Of the six proteins previously observed to be associated with cognitive impairment/dementia, four were associated with MMSE trajectories (Table 2; Figure 2A through F). One protein, MSTN (βintx = 0.08 ± 0.02, $P = 8.6 \times 10^{-6}$), the concentration of which was lower in subjects with cognitive impairment or dementia, was associated with slower decline in MMSE. Three proteins, TFF3 (βintx = −0.14 ± 0.02, $P = 2.4 \times 10^{-13}$), PI3 (βintx = −0.11 ± 0.02, $P = 4.8 \times 10^{-9}$), and PAPPA (βintx = −0.08 ± 0.02, $P = 2.1 \times 10^{-5}$) that were found in higher concentrations in subjects with cognitive impairment or dementia were associated with faster decline in MMSE.

3.3 | Proteomic profiles in serum samples from dementia cases and dementia endophenotypes

To replicate findings for the six proteins identified in the InCHIANTI analyses, we explored associations between serum protein concentration in AD subjects at the time of symptom onset with controls in the 141 subjects (74 cases, 67 controls) from BLSA and 42 subjects (25 case, 17 controls) in ROS (supplementary Table S3). In BLSA, we found a trend for lower MSTN in concurrent AD (odds ratio [OR] 0.30, 95% confidence interval [CI] 0.08 to 1.13, $P = .076$) and in ROS a trend for lower concentration of ITGAV/ITGB5 (OR: 0.15, 95% CI: 0.02 to 1.16, $P = .069$) with concurrent AD (supplementary Table S4). To further explore validation of the six proteins as putative biomarkers of dementia and cognitive impairment, we examined the associations of serum protein levels with dementia endophenotypes: Amyloid burden and brain atrophy in 146 BLSA subjects (Table 2). For MSTN, there was a marginally decreased odds of being PiB positive (b = −1.99, $P = .066$). Increased serum concentration of protein ITGAV/ITGB5 was associated with decreased global amyloid burden (mean cortical DVR; Beta: −0.21, $P = .040$) and decreased amyloid accumulation in the precuneus (Beta: −0.25, $P = .036$) in PiB+ individuals. In cross-sectional analysis of brain imaging data, we found that greater concentrations of three proteins associated with increased odds of dementia/cognitive impairment (PI3, PAPPA, AGRP) were associated with smaller brain volumes ($P < .05$) across multiple regions including amygdala, occipital white matter, entorhinal cortex, hippocampus, and parahippocampal gyrus. We found that greater concentrations of two

TABLE 2 Proteins associated with cognitive status and trajectories of cognitive decline

	InCHIANTI cross sectional cognitive status		InCHIANTI MMSE trajectory		PiB ±		Cortical DVR amyloid		Precuneus DVR amyloid		Brain volume			Brain atrophy		
Proteins	OR (95% CI)	FDRq	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P	Brain region[a]	β (SE)	P	Brain region[a]	β (SE)	P
MSTN	0.57 (0.44, 0.73)	0.004	0.08 (0.02)	8.56×10^{-6}	−1.99 (1.08)	.066	0.17 (0.14)	.236	0.18 (0.17)	.286	gm	33.2 (10.5)	.002	hip	0.05 (0.02)	.021
											tb	49.3 (17.8)	.006			
											frngm	9.66 (4.40)	.028			
											pargm	4.99 (2.44)	.041			
ITGAV/ITGB5	0.58 (0.46, 0.74)	0.007	−0.02 (0.02)	0.206	0.29 (0.66)	.663	−0.21 (0.10)	.040	−0.25 (0.12)	.036	gm	17.3 (6.84)	.011	ent	0.04 (0.02)	.015
											temgm	3.30 (1.67)	.049	pargm	−0.41 (0.20)	.040
														vcsf	−0.43 (0.21)	.041
PI3	1.78 (1.40, 2.27)	0.004	−0.11 (0.02)	4.85×10^{-9}	−0.93 (0.61)	.128	−0.05 (0.08)	.516	−0.00 (0.10)	.970	phg	−0.36 (0.13)	.005	vcsf	0.45 (0.22)	.038
											ent	−0.31 (0.12)	.007			
											am	−0.12 (0.05)	.011			
											hip	−0.35 (0.15)	.016			
TFF3	1.72 (1.32, 2.23)	0.015	−0.14 (0.02)	2.38×10^{-13}	0.98 (0.97)	.312	−0.18 (0.14)	.209	−0.18 (0.17)	.313						
PAPPA	1.68 (1.30, 2.18)	0.019	−0.08 (0.02)	2.08×10^{-5}	0.08 (0.53)	.877	−0.05 (0.06)	.399	−0.09 (0.07)	.237	hip	−0.28 (0.13)	.034			
AGRP	1.55 (1.23, 1.94)	0.035	−0.03 (0.02)	.126	−0.18 (0.99)	.857	−0.13 (0.13)	.348	−0.17 (0.16)	.294	am	−0.16 (0.08)	.043	vcsf	1.38 (0.28)	<.001
											owm	−2.68 (1.36)	.050			

[a]Abbreviations: am, amygdala; ent, entorhinal cortex; frngm, frontal gray matter; gm, gray matter; hp, hippocampus; owm, occipital white matter; pargm, parietal gray matter; phg, parahippocampal gyrus; tb, total brain; temgm, temporal gray matter; vcsf, ventricle.

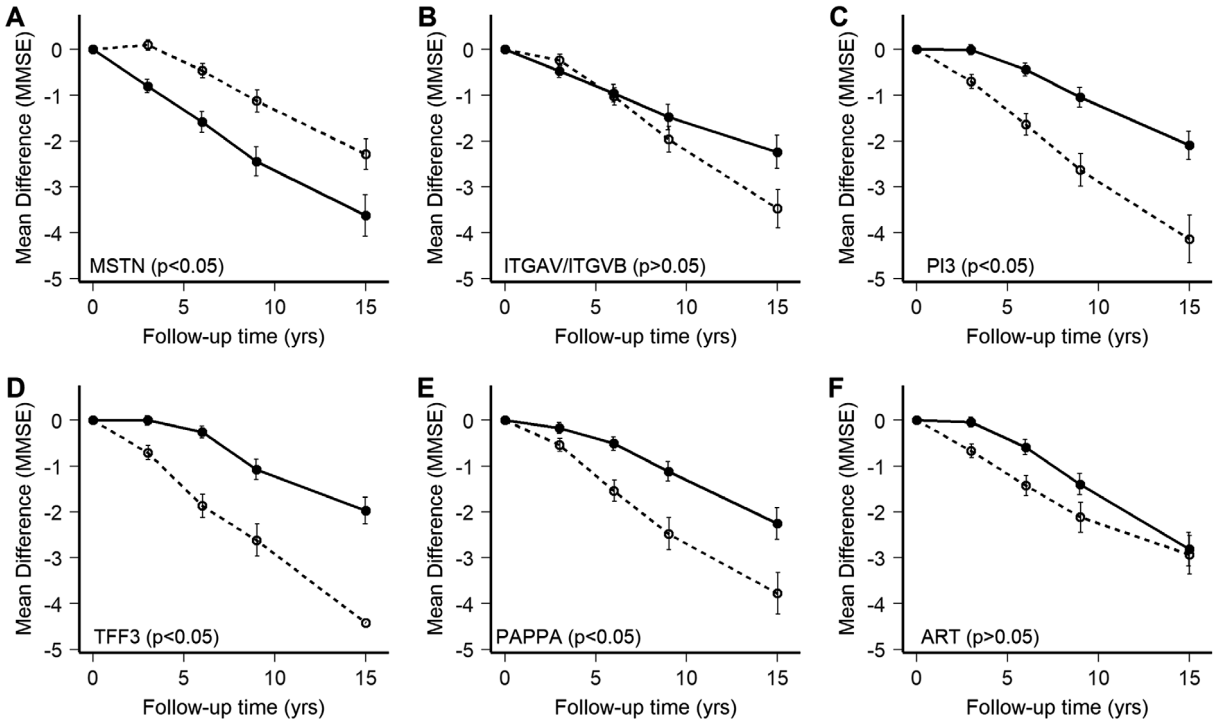

FIGURE 2 MMSE trajectory by plasma protein abundances. Line plot displaying change in MMSE (mean and standard error) over a 15-year follow-up period by baseline plasma levels of (A) myostatin (MSTN), (B) integrin aVb5 (ITGAV/ITGVB), (C) peptidase inhibitor 3 (PI3), (D) trefoil factor 3 (TFF3), (E) pregnancy associated plasma protein A (PAPPA), and (F) agouti-related peptide (AGRP). Observations are grouped by median protein abundances into high (circle) or low (triangle) plasma protein groups

proteins (MSTN, ITGAV/ITGB5) associated with a lower risk of cognitive impairment/dementia were associated with larger brain volumes ($P < .05$) across multiple regions including the total brain volume, temporal gray matter, gray matter, frontal gray matter, and parietal gray matter (Table 2). Longitudinally, we found that higher abundance of two risk proteins (AGRP, PI3) at baseline was associated with faster atrophy (ventricular expansion). The two protective proteins MSTN and ITGAV/ITGB5 were associated with slower atrophy (hippocampus, ventricle, entorhinal cortex). ITGAV/ITGB5 was also associated with faster atrophy in one brain region (parietal gray matter).

4 | DISCUSSION

Using a large population-based cohort (InCHIANTI) and two independent validation studies (BLSA and ROS) we identified a set of novel circulating proteomic biomarkers of cognitive impairment and dementia. We identified six proteins MSTN, PI3, ITGAV/ITGB5, TFF3, PAPPA, and AGRP that discriminate between subjects with normal cognitive function and those with cognitive impairment or dementia. Of interest, four (MSTN, PI3, TFF3, PAPPA) of the six proteins were also predictive of subsequent trajectories of cognitive performance over a 15-year period in older individuals who were cognitively normal at baseline. Five of these proteins (MSTN, ITGAV/ITGB5, PI3, PAPPA, and AGRP) were additionally associated with amyloid accumulation and brain atrophy in two independent validation cohorts. It is important to emphasize that the candidate proteins identified in this study show consistency across three well-characterized independent cohorts and across different endophenotypes of AD. The directions of the associations were consistent; proteins that were associated with increased odds of having dementia or cognitive impairment were predictive of faster declines in cognitive performance, smaller brain volume, and faster brain atrophy. Conversely, proteins that were associated with lower odds of having dementia or cognitive impairment were associated with a slower decline in cognitive function, lower amyloid burden, larger brain volume, and slower brain atrophy. These proteins should be further explored as candidate biomarkers of cognitive function in independent studies and, if confirmed, they may be useful in identifying persons at risk for developing dementia or cognitive impairment before the onset of overt symptoms.

Four proteins—PI3, TFF3, PAPPA, and AGRP—were associated with an increased odds of having cognitive impairment, and except for AGRP, all were associated with faster decline in cognitive function over time. PI3 had the most significant association with dementia and cognitive impairment in the InCHIANTI study, and associated with smaller brain volumes in parahippocampal gyrus, entorhinal cortex, amygdala, and hippocampus, and with faster brain atrophy in the BLSA. PI3 is a serine protease inhibitor with anti-bacterial, anti-inflammatory properties and plays a role in innate immunity.[29] Dysregulation of PI3 has been associated with several inflammatory diseases and cancer.[30,31] It is possible that PI3 influences cognitive function through the regulation of inflammation, a hypothesis that requires further exploration.

TFF3 is an intraepithelial secretory protein that is expressed primarily in the gastrointestinal (GI) tract.[32] TFF3 plays an important role in the maintenance of GI mucosal integrity and protection of the mucosal barrier.[32,33] Higher serum TFF3 has been reported in GI-related diseases such as gastric cancer[34] and inflammatory bowel disease.[35] Of interest, TFF3 is also expressed in different regions of the brain including the hippocampus, hypothalamus, and cerebellum.[36-38] In mouse models, administration of TFF3 peptide enhanced recognition and learning[39] and reduced depressive-like behavior.[40] In humans, there were no differences in TFF3 abundance in cerebrospinal fluid (CSF) between controls and subjects with mild cognitive impairment (MCI) or AD; however, lower TFF3 was associated with faster brain atrophy in amyloid-positive subjects.[41] In another study, serum TFF3 was lower in subjects with Parkinson's dementia.[42] Although these prior studies suggest TFF3 as a protective biomarker, in our study, TFF3 was a risk protein where plasma TFF3 was over-represented in subjects with dementia and cognitive impairment. It is also possible that TFF3 is not related to pathology but is an indicator of a resilience strategy aimed at controlling the primary pathological mechanism. Thus, depending on the condition, the association may be either positive or negative. Differences in results may also be due to differences in tissue (CSF vs plasma), disease stage, or underlying pathophysiology of Parkinson dementia and vascular dementia.

PAPPA is an insulin-like growth factor–binding protein proteolytic enzyme that may serve as a local regulator of insulin-like growth factor (IGF) availability.[42] PAPPA is an important prenatal biomarker of Down syndrome in the first trimester.[43] Decline in IGF levels in the brain has been implicated in the development of AD[44]; thus, PAPPA may play a role in development of AD through the regulation of IGF levels in the brain.[45] Using a supervised machine learning algorithm in the Alzheimer's Disease Neuroimaging Initiative database (ADNI) study, plasma PAPPA was one of the proteins that discriminated between cognitively normal, MCI, and AD subjects.[45] In the ADNI, higher PAPPA levels were found in MCI but were lower in AD compared to controls. In our study, PAPPA abundances increase in both cognitively impaired and dementia subjects. In addition to association with prevalence dementia and cognitive impairment, PAPPA was associated with smaller hippocampal volume in the BLSA. This may indicate that PAPPA levels may differ depending on the stage of cognitive decline. Further studies are needed to explore this possibility. Finally, AGRP was associated with smaller brain volume in the amygdala and occipital white matter, and with faster decline in global brain volume. AGRP is an appetite-simulating neuropeptide produced by the hypothalamus. Changes in eating patterns are one of the criteria for diagnosis of behavioral variant frontotemporal dementia (bvFTD). Serum AGRP was found at higher concentrations in subjects with bvFTD compared to healthy controls.[46] This is consistent with the results of the current study where plasma AGRP was overrepresented in subjects with cognitive impairment and dementia suggesting, that AGRP may serve as a general marker for dementia.

Myostatin (MSTN) and integrin $\alpha v\beta 5$ (ITGAV/ITGB5) were associated with a reduced odds of having cognitive impairment and slower decline in cognitive function over time. MSTN was further associated with larger brain volume in total brain, frontal gray matter, and parietal gray matter, in addition to showing slower hippocampal atrophy. Myostatin is a negative regulator of skeletal muscle mass.[47] There is growing evidence that age-related muscle strength is an independent risk factor for cognitive dysfunction[48] and that there may be a common pathway between functional decline in the brain and muscle. In mouse models, knocking down myostatin was associated with improved muscle function and memory.[49] Consistent with this observation, in our study, myostatin was protective of cognitive function. Integrin $\alpha v\beta 5$ was associated with larger gray matter, temporal gray matter, and slower atrophy in entorhinal cortex and total brain, and faster atrophy in the parietal gray matter. Integrin $\alpha v\beta 5$ is a dimeric transmembrane protein consisting of integrin alpha V (ITGAV) and integrin beta 5 (ITGB5) that are important regulators of cell survival and differentiation.[50] Integrin avb5 has been implicated in early development of brain metastasis. Integrin may also play an important role in neuroregulation in AD, as evidenced by higher expression of integrin avb5 in brain microvessels isolated from patients with AD compared to controls.[51] Results from the current study suggest that higher circulating levels of ITGAV/ITGB5 may be a marker of reduced dementia risk. Future studies should investigate the relationship between brain and peripheral expression of ITGAV/ITGB5 and the possible utility of this protein as a biomarker of dementia.

There has been one prior discovery study of proteomic biomarkers of AD and three studies examining the endophenotypes of AD using the SOMAscan.[7-9,52] However, there is little consistency between study results most likely due to differences in phenotypes that were analyzed. In previous proteomic studies of AD and AD endophenotypes using the aptamer-based method, the top proteins reported were not the same as those found in our analysis, and the proteins identified in our study were not among the top signals in prior studies. However, we report consistent observations in our current study across several dementia phenotypes and endophenotypes and across three independent cohorts. We find that proteins associated with a higher risk of cognitive status were associated with a higher likelihood of being PiB+ and smaller brain volume. Although the protective proteins were not associated with amyloid burden, they were associated with larger brain volumes, including the amygdala and hippocampus. Taken together, these observations provide support for the role of these six proteins as biomarkers of brain function and merit further confirmation in other studies.

Although we have identified several candidates for dementia and cognitive impairment, there are several important limitations to this study. The first is that the results of this study need to be confirmed using proteomic data generated using a different assessment method. The aptamer-based method is a fairly new technology, and concordance with proteins assessed using other method should be tested.[53] In this regard, although the proteomic data were not confirmed using an independent method in the InCHIANTI study, many of the proteins assessed by SOMAscan has been confirmed using antibody-based methods such as the Olink technology in other studies.[54] In a study examining the genetic architecture of the proteome, the protein quantitative trait loci for trefoil factor 3 (TFF3), elafin (PI3), and

agouti-related peptide (AGRP) were confirmed using the Olink assay.[54] The SOMAscan provides proteomic concentration in relative florescent units; thus, we cannot make comparisons between proteins. Furthermore, although the current aptamer-based platform measures over 1300 proteins, the assay does not profile all the proteins in the plasma. It is possible that other plasma proteins may contribute to the development and progression of cognitive impairment/dementia pathology. With rapid advancements in proteomic technology, assays that measure more proteins such as the most recent aptamer assay that measures over 5000 proteins will be critical to expand upon research presented in this report. Another limitation is that the analysis did not distinguish between different subtypes of dementia, and the discovery analysis was limited to a cross-sectional analysis. When dementia status is adjudicated in the follow-up period in the InCHIANTI study, a prospective analysis will be performed. In the longitudinal analysis of cognition, because incident dementia is unknown, the proteins associated with MMSE trajectory may not be specific to dementia and may be a more general marker of cognitive decline in aging. In addition, there were differences in the phenotype definition. In the InCHIANTI study, the primary outcome was all-cause dementia, whereas in both validation studies (BLSA, ROS) the outcome was AD. Our study has many strengths. First, the study was conducted on a large cohort of well-characterized participants that is a representative sample of the general population. Second, we show that proteins with cross sectional association with cognitive status is predictive of cognitive decline, providing evidence that these proteins may be useful in identifying individuals at risk long before the onset of symptoms. Finally, we provide internal validation with consistent association across various dementia phenotype and endophenotypes within two independent cohorts.

In conclusion, using aptamer-based proteomic profiling, we identified six candidate plasma proteomic predictors of cognitive function, and showed consistent associations with distinct dementia endophenotypes in two independent cohorts including cognitive decline, amyloid burden, and brain atrophy. These proteins may be a useful tool in identifying individuals at risk for cognitive decline and at higher risk for developing cognitive impairment and dementia. Although validation of our results is of utmost importance, our study discovered candidate proteins that could be used to identify older persons at high risk of developing accelerated cognitive decline and guide research on the molecular pathways that could be targets for interventions aimed at slowing the progression of cognitive impairment.

ACKNOWLEDGMENTS

The InCHIANTI study baseline (1998-2000) was supported as a "targeted project" (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (NIA; Contracts: 263 MD 9164 and 263 MD 821336); The InCHIANTI Follow-up 1 (2001-2003) was funded by the U.S. National Institute on Aging (contracts: N.1-AG-1-1 and N.1-AG-1-2111); the InCHIANTI Follow-ups 2 and 3 studies (2004-2010) were financed by the U.S. National Institute on Aging (Contract: N01-AG-5-0002), supported in part by the Intramural Research Program of the National Institute on Aging, National Institutes of Health, Baltimore, Maryland. ROS was supported by NIA grants P30AG10161 and R01AG15819.

CONFLICT OF INTEREST

None to declare.

DATA AVAILABILITY

Access to proteomic data is available upon review and approval of proposals submitted through the InCHIANTI study website (inchiantistudy.net). ROS data and biospecimens can be obtained by request at www.radc.rush.edu.

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SUPPORTING INFORMATION

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How to cite this article: Tanaka T, Lavery R, Varma V, et al. Plasma proteomic signatures predict dementia and cognitive impairment. *Alzheimer's Dement*. 2020;6:e12018. https://doi.org/10.1002/trc2.12018