Published in final edited form as:
Sci Transl Med. 2023 July 19; 15(705): eadf5681. doi:10.1126/scitranslmed.adf5681.

Proteomics analysis of plasma from middle-aged adults identifies protein markers of dementia risk in later life

Keenan A. Walker[1,*], **Jingsha Chen**[2], **Liu Shi**[3], **Yunju Yang**[4], **Myriam Fornage**[4], **Linda Zhou**[2], **Pascal Schlosser**[2], **Aditya Surapaneni**[2], **Morgan E. Grams**[2,5], **Michael R. Duggan**[1], **Zhongsheng Peng**[1], **Gabriela T. Gomez**[6], **Adrienne Tin**[7], **Ron C. Hoogeveen**[8], **Kevin J. Sullivan**[9], **Peter Ganz**[10], **Joni V. Lindbohm**[11], **Mika Kivimaki**[12,13], **Alejo J. Nevado-Holgado**[14], **Noel Buckley**[14], **Rebecca F. Gottesman**[15], **Thomas H. Mosley**[9], **Eric Boerwinkle**[16], **Christie M. Ballantyne**[8], **Josef Coresh**[2,*]

[1]Laboratory of Behavioral Neuroscience, National Institute on Aging, Intramural Research Program, Baltimore, MD 21224, USA.

[2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21210, USA.

[3]Novo Nordisk Research Centre Oxford (NNRCO), Oxford OX3 7FZ, UK.

[4]Brown Foundation Institute of Molecular Medicine, McGovern Medical School and Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston, Houston, TX 77030, USA.

[5]Division of Nephrology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21210, USA.

[6]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21210, USA.

[*]Corresponding author. keenan.walker@nih.gov (K.A.W.); coresh@jhu.edu (J.C.).

Author contributions: K.A.W., L.S., M.F., M.K., R.F.G., T.H.M., and J. Coresh conceptualized and designed the study. A.J.N.-H., N.B., E.B., R.C.H., C.M.B., R.F.G., T.H.M., and J. Coresh contributed to the data acquisition. K.A.W., J. Chen, L.S., Y.Y., L.Z., P.S., M.R.D., Z.P., G.T.G., and J.V.L. analyzed the data. K.A.W., L.S., Y.Y., P.S., A.S., M.E.G., M.F., A.T., P.G., R.F.G., and J. Coresh contributed to the interpretation of data. K.A.W., L.S., Y.Y., M.F., P.S., M.E.G., M.R.D., G.T.G., A.T., R.C.H., K.J.S., P.G., J.V.L., M.K., R.F.G., C.M.B., and J. Coresh drafted and provided substantial revision of the manuscript.

Supplementary Materials
This PDF file includes:
Materials and Methods
Figs. S1 to S15
References (50–106)
Other Supplementary Material for this manuscript includes the following:
Data file S1
MDAR Reproducibility Checklist

Competing interests: R.C.H. has received research grants (to his institution) from Denka Seiken and is a consultant for Denka Seiken. PG serves on the medical advisory board of SomaLogic for which he accepts no financial remuneration. A.J.N.-H. receives research funding from Janssen Pharmaceuticals, Ono Pharma, and GlaxoSmithKline. J. Coresh is a scientific advisor receiving fees from SomaLogic and Healthy.io. The other authors declare that they have no competing interests.

Data and materials availability: ARIC proteomics data are available through the National Heart Lung and Blood Institutes (NHLBI) Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) at the following link: https://biolincc.nhlbi.nih.gov/studies/aric/(accession number: HLB00020023a), and detailed study design and flowchart are provided in fig. S1. For information on how to access available data and study protocols for the ARIC study, see https://sites.cscc.unc.edu/aric/. The code used in this study can be accessed at the following repository: https://zenodo.org/record/7992838 (48).

[7]MIND Center and Division of Nephrology, University of Mississippi Medical Center, Jackson, MS 39216, USA.

[8]Section of Cardiovascular Research, Department of Medicine, Baylor College of Medicine, Houston, TX 77030, USA.

[9]Department of Medicine, Division of Geriatrics, University of Mississippi Medical Center, Jackson, MS 39216, USA.

[10]Department of Medicine, University of California-San Francisco, San Francisco, CA 94115, USA.

[11]Broad Institute of the Massachusetts Institute of Technology and Harvard University, Cambridge, MA 02142, USA.

[12]Department of Mental Health of Older People, Faculty of Brain Sciences, University College London, London WC1E 6BT, UK.

[13]Clinicum, Faculty of Medicine, University of Helsinki, Helsinki 00100, Finland.

[14]Department of Psychiatry, University of Oxford, Oxford OX1 2JD, UK.

[15]National Institute of Neurological Disorders and Stroke, Intramural Research Program, Bethesda, MD 20892, USA.

[16]Department of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas Health Science Center at Houston; Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA.

Abstract

A diverse set of biological processes have been implicated in the pathophysiology of Alzheimer's disease (AD) and related dementias. However, there is limited understanding of the peripheral biological mechanisms relevant in the earliest phases of the disease. Here, we used a large-scale proteomics platform to examine the association of 4877 plasma proteins with 25-year dementia risk in 10,981 middle-aged adults. We found 32 dementia-associated plasma proteins that were involved in proteostasis, immunity, synaptic function, and extracellular matrix organization. We then replicated the association between 15 of these proteins and clinically relevant neurocognitive outcomes in two independent cohorts. We demonstrated that 12 of these 32 dementia-associated proteins were associated with cerebrospinal fluid (CSF) biomarkers of AD, neurodegeneration, or neuroinflammation. We found that eight of these candidate protein markers were abnormally expressed in human postmortem brain tissue from patients with AD, although some of the proteins that were most strongly associated with dementia risk, such as GDF15, were not detected in these brain tissue samples. Using network analyses, we found a protein signature for dementia risk that was characterized by dysregulation of specific immune and proteostasis/autophagy pathways in adults in midlife ~20 years before dementia onset, as well as abnormal coagulation and complement signaling ~10 years before dementia onset. Bidirectional two-sample Mendelian randomization genetically validated nine of our candidate proteins as markers of AD in midlife and inferred causality of SERPINA3 in AD pathogenesis. Last, we prioritized a set of candidate markers for AD and dementia risk prediction in midlife.

INTRODUCTION

Despite advances over the past few decades, the biology of Alzheimer's disease (AD) and related dementia remains poorly understood. Within the central nervous system (CNS), deposition and aggregation of amyloid-β (Aβ) and tau neurofibrillary tangles have been identified as key features of AD. However, AD genome-wide association studies (GWAS) suggest a complex biology that extends beyond amyloid and tau accumulation (1). In addition, results from clinical and translational research indicate that systemic factors and biological processes outside the CNS can influence the risk for dementia and AD specifically (2). Support for the role of systemic factors in neurodegenerative disease comes from multiple lines of evidence, including human studies that show that systemic disease can influence the risk for AD and all-cause dementia (3, 4). In addition, translational heterochronic parabiosis studies in mice show that blood from aged mice can promote cognitive decline and microglial activation and impair neurogenesis when administered to young mice (2, 5). In support of these findings, large-scale proteogenomic studies have identified systemic circulating factors, namely, proteins, as drivers of complex CNS diseases (6, 7).

Increasingly, it is recognized that Aβ and tau represent two components of a highly complex and heterogeneous disease process (8) and that AD often co-occurs with other molecular and vascular pathology also known to contribute to cognitive decline (9). Although plasma biomarkers for amyloid/tau/neurodegeneration (A/T/N) have been established, there is ample need to identify plasma biomarkers of other disease pathways relevant to AD and related dementias. These efforts are especially important given that interventions recently shown to modify disease progression by removing cortical Aβ so far suggest only modest clinical benefit (10). Recently, a study by our group found a robust plasma proteomic signature associated with the development of dementia over a 5-year follow-up period in older adults (11). Although these efforts led to the identification of candidate markers and identified multiple proteins as being potentially causally relevant, this study was limited by its focus on the late-life proteome. The pathogenesis of AD is now understood to begin at least one to two decades before onset of clinical symptoms, a period that often coincides with middle adulthood (defined here as age 45 to 65). To understand which peripheral biological pathways are mechanistically relevant in the earliest phase of neurodegenerative disease and to identify potential pathway-specific protein markers for early dementia risk stratification, it is necessary to identify proteins and coregulated protein networks that are abnormally expressed in middle-aged adults who then develop dementia in subsequent decades.

With the exception of one recently published study (12), large-scale proteomic analyses of dementia—including a previous study published by our group—have focused on identifying risk proteins in older adults (11). Many of these studies have used cross-sectional designs, making it difficult to interpret the relevance of proteins at various stages of a protracted preclinical disease process. To address these limitations, the present study used a large-scale proteomics platform to examine the plasma proteomic signature of dementia risk in middle-aged adults followed over a 25-year period. Leveraging cross-sectional protein measurements and follow-up neurocognitive assessments from three cohorts, we identified

dementia-associated proteins involved in proteostasis, immunity, synaptic function, and extracellular matrix organization. We demonstrated that a proportion (38%) of these plasma proteins were associated with cerebrospinal fluid (CSF) $A\beta_{42}$ and p-tau as well as neurodegeneration and neuroinflammation and that 25% were abnormally expressed in patients with biomarker-defined AD. We found that many of the midlife dementia-associated proteins were differentially expressed in AD postmortem brain tissue. Using pathway and network analyses, we demonstrated that these proteins were enriched for proteostasis/proteolysis, immune, and vascular pathways, and we identified discrete midlife protein networks that were associated with dementia risk. Last, we used bidirectional two-sample Mendelian randomization to identify midlife plasma proteins that could potentially play a mechanistic role in AD risk, and we prioritized plasma protein markers of dementia risk in middle-aged adults based on the totality of multimodal evidence.

RESULTS

Midlife plasma proteins are associated with 25-year dementia risk

The study design is illustrated in fig. S1. We first examined the relationship between the abundance of 4877 plasma proteins [measured from blood drawn at Atherosclerosis Risk in Communities (ARIC) study visit 3; 1993–1995] and dementia risk over a 25-year follow-up period (median: 20.1 years). A total of 10,981 participants were included in this discovery analysis [baseline age: 60 years (SD 6); 54% women; 21% Black], 1874 (17%) of whom developed dementia before the end of the follow-up period (ARIC visit 6, 2016–2017). A detailed study flowchart and participant characteristics are provided (fig. S2 and table S1).

Unadjusted analyses found that 452 proteins measured at baseline were significantly associated with 25-year dementia risk at a Bonferroni-adjusted significance of $P < 1.03 \times 10^{-05}$ (0.05/4877 plasma proteins). After adjustment for demographic characteristics, *APOE* ϵ4 status, baseline estimated glomerular filtration rate (eGFR), and cardiovascular risk factors, 26 proteins maintained a significant association at the Bonferroni-corrected threshold (Fig. 1A and table S2). GDF15, a protein involved in metabolic and immunoregulatory function, demonstrated the strongest association with dementia risk (fig. S3). The other 25 dementia-associated proteins are known to play a role in neuronal/synaptic function (CLSTN3, CPLX1, CPLX2, GLUL, GRID2, and PSIP1), innate and adaptive immune signaling (CRLF1, FCRL4, and LEFTY2), ubiquitination and autophagy (DNJB9, DNAJB12, GABARAPL1, and HSPA1B), extracellular matrix (ECM) organization/proteolysis (ADAMTSL2, FBLN5, MMP19, and MMP12), and coagulation (F8). A list of protein names and abbreviations is provided in table S2; protein-specific biology is represented in Fig. 1B and detailed in table S3.

We next examined discrete follow-up intervals to determine whether a distinct set of midlife proteins was associated with near-term dementia risk (dementia occurring within 15 years of protein measurement, when neuropathology was likely already present) and long-term dementia risk (dementia occurring after a 15-year period, capturing proteins that are likely altered very early in the disease course). Analysis of near-term dementia risk found GDF15 and six additional midlife proteins associated with 15-year dementia risk (Fig. 1, C and D, and table S4). This included proteins involved in neuronal/synaptic function

(CBLN4), immunity (GHR and SERPINA3), growth factor binding (IGFBP2 and EGFR), and proteolysis (FAP). An analysis of long-term dementia risk found that six proteins were associated with dementia occurring 15 years or more after the midlife proteomic measurement (ALB, CLSTN3, DNAJB9, F8, FBLN5, and GDF15; Fig. 1E and table S5). Each of these proteins was also associated with dementia risk over the full follow-up time (Fig. 1, C and F). Together, we identified 32 dementia-associated proteins at this midlife period, the majority of which maintained robust associations with dementia risk when analyses were restricted to participants in their 40s and 50s at the time of blood draw for protein measurement ($n = 5285$; Fig. 1G and table S6). In sex-stratified analyses, the majority of dementia-associated proteins had similar associations in men and women (table S6). However, there were several proteins, including synaptic protein CLSTN3 and immune protein SERPINA3, which demonstrated a much stronger associations with dementia risk in men, and adhesion/ECM protein FBLN and growth factor IGFBP2, which showed a much stronger association with dementia risk in women. At least 3 of the 32 proteins—EGFR, MMP12, and SERPINA3—have been genetically linked to AD (13–15), and several genes that code for identified dementia-associated proteins overlap with GWAS risk variants for schizophrenia, intelligence, and related traits (Fig. 2A). Four of the identified proteins have been previously nominated by Accelerating Medicine Partnership (AMP) as prioritized therapeutic targets for AD (CPLX1, GABARAPL1, HTRA1, and SERPINA3) (16). Eight of the identified proteins are targeted by known drugs (table S7).

Using the set of proteins associated with dementia risk at $P < 0.01$, we conducted biological (canonical) pathway analyses to determine which biological processes and molecular functions in midlife were altered in individuals who developed dementia in subsequent decades. Platelet-derived growth factor, interleukin-3 (IL-3) signaling, and Janus kinase–signal transducer and activator of transcription (JAK/STAT) signaling were top activated pathways in individuals who developed dementia within 15 years. By comparison, inhibition of matrix metalloproteases, the unfolded protein response, bile acid biosynthesis, and granulocyte adhesion and diapedesis were among the top pathways implicated in individuals at risk for dementia beyond 15 years (Fig. 2, B and C, and tables S8 to S10). These findings implicate specific immune and vascular pathways; provide further support for the role of proteostasis, ECM activation, and other processes; and suggest that the peripheral biological pathways altered within 15 years of dementia onset are distinct from the pathways altered in earlier phases of the disease process.

Midlife dementia-associated proteins are replicated in multiple cohorts

To determine the stability of our findings across age ranges, we examined whether the midlife dementia-associated proteins maintained an association with dementia risk when measured during late-life in a subset of 4110 participants from the ARIC midlife analysis who remained non-demented 18 years after the midlife protein measurement (table S11). This internal replication related the 32 candidate proteins measured at ARIC visit 5 (2011–13) to incident dementia occurring over the final 5 years of the full 25-year follow-up (median follow-up, 4.9 years; 428 incident dementia cases) (11). In this analysis, 25 of the 32 (78%) candidate proteins maintained a significant association with dementia risk when

measured during late-life [false discovery rate (FDR)–corrected $P < 0.05$] (Fig. 3A and table S12).

To determine whether the candidate proteins were differentially expressed in individuals with AD dementia, we next used data from the European Medical Information Framework for Alzheimer's Disease (EMIF-AD) study: a cohort of 972 participants classified as having AD dementia or mild cognitive impairment (MCI) or cognitively normal (table S13). Twenty-two of the 32 candidate proteins were measured in plasma of EMIF-AD participants. Twelve of these proteins (54%) were either differentially expressed in AD dementia (versus controls) or associated with conversion from MCI to AD dementia at $P < 0.05$ over 2-year follow-up. Nine proteins remained significant after correction for multiple comparisons (Fig. 3B and table S14). Four of the 12 replicated proteins demonstrated opposite associations in midlife (ARIC study, incident dementia; Fig. 3A) compared with late-life (EMIF-AD, prevalent and incident AD dementia; Fig. 3B) analyses. One synaptic protein (EPHA10) and one autophagy protein (GABARAPL1) were up-regulated during midlife in individuals at risk for dementia yet down-regulated during later life in individuals with clinically defined AD dementia. On the other hand, two synaptic proteins (GRID2 and CBLN4) were down-regulated during midlife in individuals at risk for dementia yet up-regulated during later life in individuals with prevalent or incident AD dementia.

Subtle cognitive changes can occur well before the onset of dementia and may represent the earliest clinical manifestations of a neurodegenerative disease (17). To determine which midlife dementia-associated proteins were associated with these early cognitive changes, we used data from 1834 non-demented middle-aged adults [age: 56 (SD 6)] in the Whitehall II cohort (table S15). After adjusting for demographic factors, eGFR, and *APOE*ϵ4 status, we found that 4 of the 32 dementia-associated proteins measured during midlife (DNAJB9, GDF15, HSPA1B, and MMP19) were significantly associated with cognitive decline ($P < 0.05$), although none survived correction for multiple comparisons (Fig. 3C and table S16). This subset of proteins, involved in protein ubiquitination, immune function, and ECM organization, may reflect the peripheral biology underlying some of the earliest cognitive changes preceding dementia. Together, 15 of the 32 midlife dementia-associated proteins were supported in at least one of the external replication cohorts (EMIF-AD or Whitehall II).

Midlife dementia-associated proteins correlate with CSF amyloid, p-tau, neurodegeneration, and neuroinflammation

To determine whether the identified proteins are associated with neurobiological processes specific to AD, we related plasma protein to brain amyloid (measured in either CSF or amyloid PET) and CSF p-tau in the EMIF-AD cohort. Of the 22 dementia-associated proteins measured in EMIF-AD, 7 (ALB, CBLN4, DNAJB9, FAP, F8, GABARAPL1, and GRID2) demonstrated associations with brain amyloid–positive status (Fig. 3D and table S17). Three of these proteins were also associated with elevated CSF p-tau, and one additional protein (MB) was associated with p-tau but not amyloid. In the same cohort, each of these proteins was differentially expressed in amyloid-positive participants with tau/neurodegeneration (A+/TN+) compared with controls (A−/TN−), supporting their relevance to AD pathogenesis (fig. S4 and table S18).

Because circulating proteins may influence dementia risk through neurodegenerative and neuro-immune pathways not specific to AD, we next examined the association of dementia-associated proteins measured in plasma with CSF markers of neurodegeneration, including total-tau (t-tau), neurofilament light chain (NfL), and neurogranin (Ng), as well as neuroinflammation (YKL-40) in the EMIF-AD cohort. We found that several proteins not associated with AD biomarkers were nonetheless significantly associated with CSF expression of NfL (MMP12, EPHA10, and GHR) and YKL-40 (GDF15) ($P < 0.05$). Our results suggest that these proteins may be involved in neurodegenerative and neuroimmune processes not specific to AD (Fig. 3D and table S17).

Brain abundance of dementia-associated proteins identified in plasma is associated with AD

Using the Genotype-Tissue Expression database and data from the Human Protein Atlas, we found that a subset of dementia-associated proteins identified in plasma were expressed in postmortem brain tissue (fig. S5). To determine whether these plasma proteins were also altered in the brains of AD patients, we computed results from a proteome-wide analysis of brain tissue: a combined cohort including the Baltimore Longitudinal Study of Aging, Banner Sun Health Research Institute, Mount Sinai School of Medicine Brain Bank, and Adult Changes in Thought Study (18). Of the 10 candidate proteins measured in brain tissue in this combined cohort, 5 (CPLX1, CPLX2, GABARAPL1, HSPA1B, and EGFR) were differentially expressed ($P < 0.05$) in the brains of patients with symptomatic AD compared with control brains. Of the 18 dementia-associated proteins measured in brain tissue within the Religious Orders Study and Rush Memory and Aging Project (19), 5 (CBLN4, CPLX1, HSPA1B, HTRA1, and SMC3) were differentially expressed in AD compared with control brain tissue (Fig. 3E and tables S19 and S20).

Midlife protein networks implicate distinct biology in near- and long-term dementia risk

We next used a data-driven approach to group plasma proteins into clusters or modules constructed based on protein coexpression patterns. Nineteen non-overlapping protein modules were identified, ranging in size from 23 to 2025 proteins (Fig. 4, A and B, and table S21). We quantified person-specific module expression and found that three modules (M1, M5, and M19) were significantly (FDR-corrected $P < 0.05$) associated with 25-year dementia risk (Fig. 4C and table S22). A distinct protein module, M9, was associated with near-term dementia risk, whereas modules M1, M5, and M19 were also associated with long-term dementia risk (Fig. 4, D and E, and fig. S6). The protein module M9 was enriched for complement and coagulation proteins (Fig. 4F) and included top near-term dementia-associated proteins GHR and SERPINA3. Tissue enrichment analysis revealed that M9 was enriched for liver proteins (fig. S7). Protein modules associated with long-term dementia risk were enriched for proteins involved in JAK-STAT signaling, cytokine signaling, T helper 1 (T_H1) and T_H2 cell differentiation (M1), ECM degradation/organization and leukocyte activation (M5), and immune/mitogen-activated protein kinase signaling and proteins regulated by the c-Jun transcriptional activator (M19) (Fig. 4, G to I). Tissue-specific enrichment analysis suggested multiple tissues of origin, including appendix (M5 and M19), lymph node (M5), and salivary gland (M5) (fig. S7). Together, these results suggest a multidecade immunologic signature characterized by early involvement of JAK-

STAT and Toll-like receptor signaling, leukocyte activation, and ECM degradation, followed by more prominent alteration of complement and coagulation protein networks later in the disease course (Fig. 5, A and B).

Proteins most correlated with overall module expression (hub proteins) tended to be differentially expressed in AD brain tissue at the RNA level in the AMP-AD RNA sequencing Harmonization Study (Fig. 4, F to I) (20). A hub protein for M9 (RET proto-oncogene) and a hub protein for M19 [GBP1 (guanylate binding protein 1)] have been previously nominated by AMP as AD therapeutic targets and independently associated with neuroimmune and other disease-relevant processes (21–23).

Genetic support for the mechanistic association between plasma proteins and AD

To infer causality between dementia-associated proteins and AD, we performed bidirectional two-sample Mendelian randomization. We conducted a GWAS of plasma protein abundance within the ARIC sample that identified independent protein quantitative trait loci (pQTLs) for 28 of the 32 dementia-associated proteins and all four dementia-associated protein networks (table S23). No plasma protein or protein network was found to be associated with AD in the forward direction at a Bonferroni-corrected significance threshold. However, there was evidence for a direct relationship between plasma abundance of SERPINA3 and CLSTN3 and risk of AD at an uncorrected threshold of $P < 0.05$ (Table 1). Median weighted sensitivity analyses further supported a potential causal link between SERPINA3 and AD (table S24). SERPINA3, also known as alpha-1-antichymotrypsin, is a peptidase inhibitor that has been previously associated with AD, AD age of onset (13), and primary progressive multiple sclerosis (24). SERPINA3 is up-regulated in the context of inflammation (25) and can promote the assembly of Aβ peptides into filaments (26). The present study provides further support for a potential mechanistic role of SERPINA3 in AD and suggests that it may be particularly relevant within 15 years of dementia onset.

Mendelian randomization analyses in the backward direction supported AD as a cause of altered plasma protein abundance for 9 of the 32 dementia-associated proteins using the inverse-variance weighting (IVW) method ($P < 0.05$) (Table 1). In sensitivity analyses, seven proteins (ABHD14A, CPLX2, GHR, IGFBP2, MMP12, NDST1, and PSIP1) showed robust associations using a threshold of $P < 0.05$, three of which remained significant at a Bonferroni-corrected threshold (table S24). These results suggest that a proportion of the dementia-associated proteins are dysregulated during midlife—potentially decades before dementia onset— as a result of biological changes accompanying AD.

Midlife dementia-associated plasma proteins are linked to diseases and traits that contribute to dementia risk

Plasma proteins not causally implicated in AD may still be mechanistically involved in dementia through non-AD pathways. Using Mendelian randomization results from the Proteome PheWAS browser (27), we found that dementia-associated proteins were implicated in several neurological phenotypes and vascular, inflammatory, and metabolic conditions that are established risk factors for dementia (Fig. 6; full results in table S25). For example, SERPINA3 and GDF15 were associated with regional brain volume;

MMP12, FCRL4, and CBLN4 were associated with ischemic stroke, large vessel disease, and coronary heart disease; and GRID2 and MMP12 were associated with inflammatory/autoimmune conditions, including rheumatoid arthritis and psoriasis.

Dementia-associated protein pQTLs are associated with brain transcriptional signature and neurobiological pathways

Several of the top dementia-associated proteins, including GDF15, were not found in postmortem brain tissue in high abundance (fig. S5). Even with low or undetectable quantities in the brain, peripherally secreted proteins and genetic regulators of these proteins (pQTLs) may still influence target cells within the CNS (28). To examine this possibility, we first examined whether dementia-associated protein pQTLs have been identified as brain tissue expression quantitative trait loci (eQTLs). We found that dementia-associated protein pQTLs are also eQTLs for 91 unique cis-genes (up to 1-Mb window) expressed in brain tissue, henceforth referred to as eGenes (table S26). Colocalization analysis supported that 77.5% of the protein-eGene pairs had shared genetic contribution (fig. S8 to 11 and table S27). Using publicly available brain proteomic and transcriptomic datasets (18, 19, 29), we found that a large proportion of eGenes (62%) and their translated protein products (33%) were differentially expressed in AD brain tissue (table S28). Thus, genetic variants that influence abundance of plasma dementia-associated proteins during midlife may also regulate expression of multiple genes that are abnormally expressed in AD brains.

Proteomic prediction of dementia risk

Last, we used data from the ARIC study to examine the prognostic utility of cross-sectionally measured midlife dementia-associated proteins for predicting all-cause dementia over the full 25-year follow-up period. We used elastic net machine learning followed by 10-fold cross validation to select the optimal weighted combination of proteins from among the 32 candidate proteins identified in the discovery analysis. As displayed in fig. S12, the protein-only prediction model incorporated 13 proteins and demonstrated an area under the ROC curve (AUC) in the validation cohort of 0.66 [95% confidence interval (95% CI): 0.65, 0.68]. By comparison, demographic factors, *APOE*ϵ4 status, and cardiovascular risk factors together predicted future dementia with an AUC of 0.77 (95% CI: 0.76, 0.78). Adding candidate plasma protein markers to the demographic/cardiovascular risk factor model showed modest, but significant, improvement in prediction accuracy (AUC, 0.78; 95% CI: 0.77, 0.79; C stat. Δ: 0.011; $P = 8.74 \times 10^{-09}$). Improved prediction with the addition of dementia-associated proteins (over that of combined demographic/clinical variables) was found for both near-term and long-term dementia risk.

DISCUSSION

Understanding the midlife proteomic signature associated with AD and dementia risk can provide insight into relevant biological pathways and facilitate identification of early-stage markers and molecular drivers of disease. The present study leveraged data from multiple cohorts to identify and characterize 32 proteins and four protein networks in plasma of middle-aged adults that were strongly associated with dementia risk in subsequent decades. The dementia-associated proteins identified in this study fall largely into one of

four overlapping biological processes: proteostasis, immunity, synaptic function, and ECM organization. As illustrated in figs. S13 and S14, these markers can be used as tools to quantify midlife perturbations in a distinct set of biological processes linked to later AD and related forms of dementia. We found only modest overlap between the dementia-associated plasma proteins identified at late life in our previous study (11) and the proteins associated with long-term dementia risk during midlife (6 of 32 proteins; CPLX2, SERPINA3, GDF15, GHR, FBLN5, and PSIP1). These results support the idea that the peripheral biological pathways associated with future dementia risk change with increasing age and disease progression.

Identified proteostasis proteins include DNJB9, DNAJB12, GABARAPL1, and HSPA1B, all of which were elevated in participants who developed dementia over the 25-year follow-up period. HSPA1B, also known as heat shock protein 70 (Hsp70), and DNAJB9/DNAJB12, co-chaperones for Hsp70 and Hsp40, play an integral role in protein quality control (PQC) and protein degradation, whereas GABARAPL1 is involved in autophagosome maturation. In addition to demonstrating that these PQC and stress response proteins are abnormally elevated in plasma decades before dementia onset, we demonstrated that DNAJB9 and GABARAPL1 are associated with brain amyloid, p-tau, and neurodegenerative and neuroinflammation markers. HSPA1B, although not available in the CSF cohort, was elevated in AD brains. We show that this up-regulation of PQC and stress response proteins, possibly in response to the accumulation of misfolded proteins in the CNS, can be detected in plasma during midlife, well before the onset of dementia. Given the early association with dementia risk, expression in brain tissue, strong correlation with AD pathology, DNAJB9, GABARAPL1, and HSPA1B may serve as markers of the proteostatic response in AD and should be investigated for their mechanistic relevance.

Supporting the growing body of evidence for the central role of immune function in neurodegenerative disease, the current study identified several immunologically relevant dementia-associated proteins, and pathway analyses implicated specific immune processes in near-term (IL-3 and JAK/Stat signaling) and long-term (granulocyte adhesion and diapedesis) dementia risk. Furthermore, we identified a dementia-associated protein module (M19) that was enriched for both immune and stress response (heat shock) proteins, suggesting that a coregulated change to the immune and stress response occurs very early in the course of dementia. GDF15, an immuno-metabolic stress response protein, demonstrated the strongest association with 25-year dementia risk. In addition to being the only midlife protein associated with nearand long-term dementia risk, higher GDF15 was also associated with midlife cognitive decline and neuroinflammation. GDF15 was not detectable in brain, nor was it associated with CSF Aβ or p-tau, suggesting that it is not an AD-specific protein. GDF15 is highly expressed by senescent cells as a core feature of the senescence-associated secretory phenotype (30), a prolonged proinflammatory cellular state associated with age-related diseases (31). Although GDF15 has been linked to adverse neurocognitive outcome previously (12, 32), our Mendelian randomization analyses did not suggest that GDF15 has a causal role in AD. However, this does not preclude its mechanistic relevance to neuroinflammation or other processes relevant, but not specific, to AD.

Our results suggest a potential causal relationship between plasma SERPINA3, an innate immune protein involved in JAK-Stat signaling, and AD. We validated the SERPINA3-AD association in an external cohort and demonstrated that this previously nominated AD therapeutic target was coexpressed with a dementia-associated network enriched for coagulation and complement proteins. Although SERPINA3 demonstrated a positive association with dementia risk and AD, Mendelian randomization suggested that elevated SERPINA3 may be protective against AD. This seemingly contradictory relationship is observed for other proteins that may play a protective role yet are secreted at higher concentrations in the context of disease progression, such as sTREM2 (33). EGFR, a cell surface protein that binds to epidermal growth factor, is another immunologically relevant dementia-associated protein identified herein. *EGFR* was recently identified as a candidate risk gene for AD in a larger GWAS (14), and previous work has implicated this protein in multiple neuropathological processes, including reactive astrocytosis, neuroinflammation, and axonal degradation (34). The robust association between midlife plasma EGFR and 15-year dementia risk provides further support for the genetic link between *EGFR* and AD and indicates that the repurposing of clinically approved EGFR drugs for treatment of AD may warrant further consideration.

Of the synaptic proteins associated with dementia risk in this study, two in particular, CBLN4 and GRID2, also demonstrated a strong positive association with amyloid status. CBLN4 is a secreted protein involved in inhibitory GABAergic synapse formation and maintenance. GWAS have linked variants on or near the *CBLN4* gene to vascular dementia (35). Genetic variation in the gene coding for GRID2, an ionotropic glutamate receptor, has been associated with intelligence (36), cognition (37), and educational attainment (38). Here, we show that these two synaptic proteins may function as midlife markers of AD and all-cause dementia. Other synaptic proteins identified here, including CPLX1 (a nominated AD therapeutic target) (29), CPLX2 (11), and CLSTN3 (39), have been previously implicated in AD as well. Several of the synaptic proteins (EPHA10, GRID2, and CBLN4) associated with dementia risk when measured during midlife demonstrated reverse association between AD dementia and CSF AD and neurodegeneration biomarkers, suggesting a multiphasic relationship between plasma synaptic proteins and dementia risk that varies by disease stage.

ECM/proteolysis proteins, including matrix metalloproteinases (MMPs) MMP12 and MMP19, were also associated with dementia risk in the current analysis. Supporting these findings, a genetic locus near *MMP12* (rs12808148) that we identified as a pQLT for plasma MMP12 has been associated with AD risk previously (15). Although our Mendelian randomization findings did not suggest that plasma MMP12 is directly implicated in AD pathogenesis, this protein has been mechanistically linked to stroke, myocardial infarction, and inflammatory conditions such as psoriasis, all of which are known dementia risk factors. If circulating ECM/proteolysis proteins do have a mechanistic role in dementia, our findings suggest that these proteins are altered early in the disease course and likely operate through vascular or immune pathways, consistent with what has been suggested previously (40).

Our study leveraged proteomic and genetic data to make inferences about the mechanistic relevance and the directionality of a relationship between dementia-associated plasma proteins and AD risk. Using proteomics and AD GWAS data, we demonstrated in

Mendelian randomization analyses that nine (28%) of the dementia-associated plasma proteins identified herein show altered abundance in plasma potentially as a result of early biological changes related to AD. We suggest that some of the identified proteins may play a compensatory rather than a direct role in AD, perhaps representing an allostatic pattern of response to preserve homeostasis during the early stages of AD. The lack of an association between plasma proteins and AD in Mendelian randomization analyses does not necessarily preclude a mechanistic role for candidate proteins in AD or other forms of dementia. Although the dementia-associated proteins alone did not provide highly accurate prediction of 25-year dementia risk (C-statistic of 0.66), these proteins, in combination, did add modest predictive value to a group of demographic and clinical variables that are themselves strong predictors of dementia risk. These findings underscore the potential added value of plasma proteins beyond traditional risk factors for stratifying patients or clinical study participants based on dementia risk.

Our study has some limitations. First, dementia was not classified on the basis of etiology in the discovery analyses. However, we used an external cohort, CSF AD biomarkers, and Mendelian randomization to demonstrate that a subset of these proteins may be relevant in AD. This was expected given that the majority of dementia cases in our community sample likely have a mixed/AD pathology based on prevalence estimates (41). Second, our discovery analyses were restricted to Black and Caucasian individuals living in the United States. Although replication of protein-dementia associations in participants from the United Kingdom supports the generalizability of our findings, the results may not extend to participants from non-Black and non-white groups within or outside of the United States. Third, to account for confounding, our analyses adjusted for several known dementia risk factors, including diabetes and kidney dysfunction, each of which may represent an intermediate factor through which protein abundance may influence dementia risk. This approach may be overly conservative, perhaps suppressing protein-dementia relationships that operate through such risk factors (42). Last, although the SomaScan proteomics platform used here provides a comprehensive measurement of the human proteome, not all circulating proteins were measured; there is a known overrepresentation of secreted proteins, which may bias findings. Despite these limitations, the current study identified a number of pathway-specific plasma proteins that may be relevant in the earliest phase of AD and related dementias.

MATERIALS AND METHODS

Study design

The objective of the current study was to discover early plasma protein markers and further understand the systemic factors that promote neurodegeneration using a large-scale proteomics approach. The current analysis used a cohort study design (detailed below) for discovery of dementia-associated proteins (ARIC cohort) and replication of protein-dementia (EMIF-AD cohort) and protein-cognitive decline (Whitehall II cohort) associations. ARIC is an ongoing community-based study that initially enrolled 15,792 participants between 1987 and 1989 from four communities within the United States: Jackson, MS; northwestern suburbs of Minneapolis, MN; Forsyth County, NC; and

Washington County, MD (43). Until visit 4 (1996–1998), participants were evaluated every 3 years at in-person study visits. Participants were brought back 15 years later for visit 5 (2011–2013) and about 5 years later for visit 6 (2016–2017). Data collection for subsequent ARIC study visits is still ongoing. No statistical methods were used to predetermine sample size for discovery and replication analyses. Sample sizes were determined on the basis of available data.

Participants were excluded from the ARIC discovery analyses if they were non-white or non-Black (due to low sample size) and from the Minnesota and Washington County studies if Black (due to low sample size). Participants with missing essential covariates, missing SomaScan protein measurement, or a dementia diagnosis on or before the study's baseline visit (visit 3) were excluded from the ARIC study (fig S2). Exclusion criteria were preestablished. A total of 10,981 participants were included in the ARIC discovery analysis. A detailed study design and flowchart is provided in fig. S2. Institutional review boards approved the study protocols at each participating center: Johns Hopkins University, Baltimore, MD; University of Mississippi Medical Center, Jackson, MS; University of North Carolina at Chapel Hill, NC; and Wake Forest University, Winston-Salem, NC. All participants gave written informed consent at each study visit, and proxies provided consent for participants who were judged to lack capacity. The current study complies with STROBE guidelines.

Protein measurements in plasma

We conducted protein measurement of blood collected at ARIC visit 3 using the SOMAmer-based capture array (44) method (SomaScan platform), which quantifies the relative concentration of plasma proteins or protein complexes. The SomaScan platform uses short single-stranded DNA with chemically modified nucleotides (modified aptamers) that act as protein-binding reagents with defined three-dimensional structures and unique nucleotide sequences. These aptamers are identifiable and quantifiable using DNA detection technology. The median intra- and interrun coefficients of variation (CV) have been previously reported to approximate 5% with intraclass correlation coefficients of ~0.90 (44). The lower limit of detection of the SomaScan assay extends into the femtomolar range, below that offered by conventional immunoassays. Assay performance characteristics have been described in detail previously (45).

Plasma was collected from each ARIC study site using a standardized protocol: frozen at −80°C and shipped on dry ice to the ARIC central laboratory, where it was continuously frozen until aliquoting into barcoded microtiter plates with screw-top lids. Plates were sent to SomaLogic for quantification. In total, 5284 modified aptamers (SOMAmer reagents or "SOMAmers") were used to quantify relative protein abundance.

SomaLogic quality control—Of the 11,564 ARIC participants with plasma samples measured, 39 were flagged excluded for failing to meet the acceptable quality control criteria. Sixty-eight plates were run for 5284 SOMAmers. The manufacturer flagged SOMAmers if the interpolate calibration factor was outside acceptable criteria (quality control ratio of 0.8 to 1.2). SOMAmers were not excluded on the basis of being flagged.

We found that 2 of the 32 dementia-associated proteins each had one flagged plate and that protein-dementia associations were similar after excluding measurements on flagged plates.

ARIC quality control—Log_2 transformation was applied to all SOMAmer measures to correct for skewness. We ran blind duplicates for 414 of the 11,564 (4%) participants. The median interassay CV for SOMAmers measured at visit 3 [calculated using the Bland-Altman method (CV_{BA})] was 6.3%. The median split sample reliability coefficient was 0.85 at visit 3, after excluding quality control outliers. ARIC quality control steps have been outlined in detail previously (11) and are described in the Supplementary Materials and Methods. A total of 4877 SOMAmers measuring 4697 unique proteins or protein complexes passed quality control and were measured in the current study. Interassay CV_{BA}s and reliability coefficients for SOMAmers associated with dementia risk in the primary analysis are provided in table S29. A comparison of CVs derived from midlife baseline protein measurements (visit 3) compared with CVs derived from blood collected 18 years later (visit 5) suggests a modest effect of storage duration on the reliability of protein measurement. However, the CVs at both visits were low, suggesting minimal measurement error.

Protein validation—We validated the SomaScan GDF15 measurement using a traditional immunoassay (Roche Diagnostics, catalog no. 07125933190) in plasma collected at ARIC visit 5. SomaScan and the Roche GDF15 assays were highly correlated ($n = 142$, $r = 0.94$). The Roche GDF15 assay was used at the recommended dilutions as per the manufacturer protocol. In total, 20 of the 32 (63%) SOMAmers binding to dementia-associated proteins have been validated previously with data-dependent analysis mass spectrometry, multiple reaction monitoring mass spectrometry, or identification of cis pQTLs using GWAS (table S30).

Covariate assessment

Participant education (less than high school/high school; general education diploma or vocational school/at least some college), race (Black/white), and sex (male/female) were reported at ARIC visit 1. Because race and study center are highly confounded, a race-study center variable was used (white-Washington County/white-Forsyth County/Black-Forsyth County/white-Minneapolis/Black-Jackson). *APOE* (coded as 0 *APOE*e4 alleles/≥1 *APOE*e4 alleles/missing) was genotyped using the TaqMan assay (Applied Biosystems). All other covariates [eGFR, hypertension, diabetes, body mass index (BMI), and smoking status] were assessed at visit 3, concurrent with plasma proteomic measurement (see Supplementary Materials and Methods for detailed description).

Dementia ascertainment

The discovery analysis included 10,981 ARIC participants [mean age 60 years (SD 6); 54% women; 21% Black]. As illustrated in fig. S2, participants underwent a limited cognitive assessment at visit 2 (1990–1992) before the baseline protein measurement (visit 3), another cognitive exam at visit 4 (1996–1998), and more comprehensive cognitive and functional exam at visit 5 (2011–2013) and visit 6 (2016–2017). For participants who, because of death or visit nonattendance, were not seen at visit 5, the Clinical Dementia Rating (CDR) scale, Functional Activities Questionnaire (FAQ), Telephone Interview for

Cognitive Status-Modified (TICSm), and hospital discharge and death certificate codes from the ARIC hospital surveillance were used to define dementia diagnosis and date of dementia onset. For participants who attended visit 5, dementia was confirmed on the basis of a comprehensive cognitive and functional exam; previously administered CDR, FAQ, and TICSm, and hospital discharge codes were used to define date of dementia onset. Dementia surveillance methods between ARIC visit 1 and visit 5 have been detailed previously (46). After visit 5, participants were administered the Six-Item Screener (SIS), a brief cognitive assessment, annually via phone. For participants who received a low score on the SIS and participants who were unable to participate in the screening via phone, the AD8 was administered to the participant's informant. For participants who did not attend visit 6, due to death or visit nonattendance, the SIS, AD8, and hospital discharge and death certificate codes were used to define dementia diagnosis and date of dementia onset. For participants who attended visit 6, dementia was confirmed on the basis of a comprehensive cognitive and functional exam; previously administered SIS and AD8 and hospital discharge codes were used to define date of onset up to 31 December 2017 (11, 47). Dementia classification was based on the NIA/AA (National Institute on Aging and Alzheimer's Association) and the Diagnostic and Statistical Manual of Mental Disorder–Fifth Edition (DSM-5) criteria. Criteria for dementia classification are detailed in the Supplementary Materials and Methods. The dementia incidence rate was 9.63 (95% CI: 9.21 to 10.08) cases per 1000 person-years.

Replication cohorts

EMIF-AD—We used data from the EMIF-AD Multimodal Biomarker Discovery study to examine the association of candidate proteins with prevalent AD dementia, progression from MCI to AD, and CSF biomarkers of AD pathology, neurodegeneration, and neuroinflammation. The EMIF-AD study includes samples from about 1200 participants representing three cognitive groups: cognitively normal controls (CTL), MCI, and AD dementia. A total of 972 participants (372 CTL, 409 MCI, and 191 AD) had plasma specimens measured using SomaLogic's SomaScan v.4 aptamer-based platform available for the current analyses. CSF was collected concurrently with the blood draw used for proteomic analyses. EMIF-AD study details and statistical analyses are described in the Supplementary Materials.

The Whitehall II study—We used data from the Whitehall II study to examine the association of midlife candidate protein abundance with subsequent 20-year cognitive decline. A total of 10,308 civil servants (age 35 to 55) in London, United Kingdom were enrolled. Blood specimens were taken from a subsample of 2274 non-demented participants from 1997 to 1999. Protein abundance was measured by applying SomaLogic's SomaScan v.4 aptamer-based platform to plasma specimens. Four clinical examinations and cognitive assessments were conducted from 2002 to 2016 after blood collection from which global cognitive scores were derived. Analyses examined the adjusted association of plasma protein abundance with cognitive decline slope. Whitehall II study details and statistical analyses are provided in the Supplementary Materials.

Statistical analysis

We used Cox proportional hazards regression models to examine the association between the abundance of 4877 proteins (SOMAmers) measured during midlife (ARIC visit 3) and incident dementia occurring between visit 3 and visit 6. After examining protein-dementia associations in an unadjusted model, we examined a model that adjusted for potential confounders, including demographic variables (age, sex, race-study center, and education), *APOE*ϵ4 status, kidney function defined as eGFR creatinine, and cardiovascular risk factors (BMI, diabetes, hypertension, and smoking status). The fully adjusted model was used for all primary analyses. A Bonferroni-corrected two-sided P value < 0.05 was used to determine statistical significance. The Cox proportionality assumption was tested by computing and plotting Schoenfeld residuals. Covariates that did not meet the proportional Hazards assumption were incorporated in sensitivity analyses as stratified variables or with a time interaction (covariate*time) to determine whether results differed from that derived in the primary analyses.

For internal replication of candidate proteins, we used late-life SomaScan protein measurements performed at ARIC visit 5 (2011–2013). Visit 5 proteins underwent quality control (QC) procedures similar to that described above for midlife proteins. The analysis of proteins in relation to dementia risk included participants with available protein measurements who were non-demented at visit 5. Full inclusion/exclusion criteria are provided in fig. S15. The same adjusted Cox proportional hazard model used in the midlife discovery analysis was applied to relate the 32 proteins measured in late-life to 5-year dementia risk through ARIC visit 6 (2016–2017). An FDR-corrected two-sided P value < 0.05 was used to determine statistical significance. Analyses were conducted using R v3.6.2 and Stata, version 14.

A detailed description of the EMIF-AD and Whitehall II studies is provided in the Supplementary Materials and Methods. Methods used for the construction of coexpression networks and Mendelian randomization methods are also described in the Supplementary Materials and Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We thank the staff and participants of the ARIC study for important contributions.

Funding:

This work was supported, in part, by the Intramural Research Program of the NIA-NIH (K.A.W., M.R.D., and Z.P.), the NINDS Intramural Research Program (R.F.G.), and NIA grants U01AG052409 and U01AG058589 (M.F.). This project was also supported by DFG Project-ID 192904750–CRC 992 Medical Epigenetics, by the DFG grant SCHL 2292/1 (Walter Benjamin Fellowship), and the EQUIP Program for Medical Scientists, Faculty of Medicine, University of Freiburg (P.S.). This project was also supported in part by NIAMS R01AR073178 (A.T.), the Päivikki and Sakari Sohlberg foundation (J.V.L.), the Virtual Brain Cloud from European commission (grant no. H2020-SC1-DTH-2018-1) (L.S.), and NIDDK R01DK124399 (M.E.G. and J.C.), R01DK108803 (M.E.G.), R01DK115534 (M.E.G.), R01DK100446 (M.E.G.), K24HL155861 (M.E.G.), and the National Kidney Foundation (M.E.G.). This work was also supported by the UK Medical Research Council (MRC S011676

and R024227/1) (M.K.), the NIA (R01AG056477) (M.K.), the Wellcome Trust (221854/Z/20/Z), and the Academy of Finland (350426) (M.K.). The ARIC Study is supported by NIH grants R01HL087641 (E.B.), R01HL059367, and R01HL086694. The ARIC Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201700001I, HHSN268201700002I, HHSN268201700003I, HHSN268201700004I, and HHSN268201700005I). Neurocognitive data are collected by U01 2U01HL096812, 2U01HL096814, 2U01HL096899, 2U01HL096902, and 2U01HL096917 from the NIH (NHLBI, NINDS, NIA, and NIDCD) and with previous brain MRI examinations funded by R01HL70825 from the NHLBI. This work was supported by NIH contract HHSN268200625226C. Infrastructure was partly supported by grant number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. SomaLogic Inc. conducted the SomaScan assays in exchange for use of ARIC data. This work was supported in part by NIH/NHLBI grant R01 HL134320. Reagents for GDF15 assays were donated by the Roche Diagnostics Corporation. The Whitehall II study is supported by the Medical Research Council (MRC R024227/1), National Institute on Aging, USA (R01AG056477), and the Wellcome Trust (221854/Z/20/Z). SomaLogic Inc. conducted the SomaScan assays for 2274 Whitehall participants in exchange for use of Whitehall data in an academic-industry partnership project between UCL, other universities, and SomaLogic Inc.

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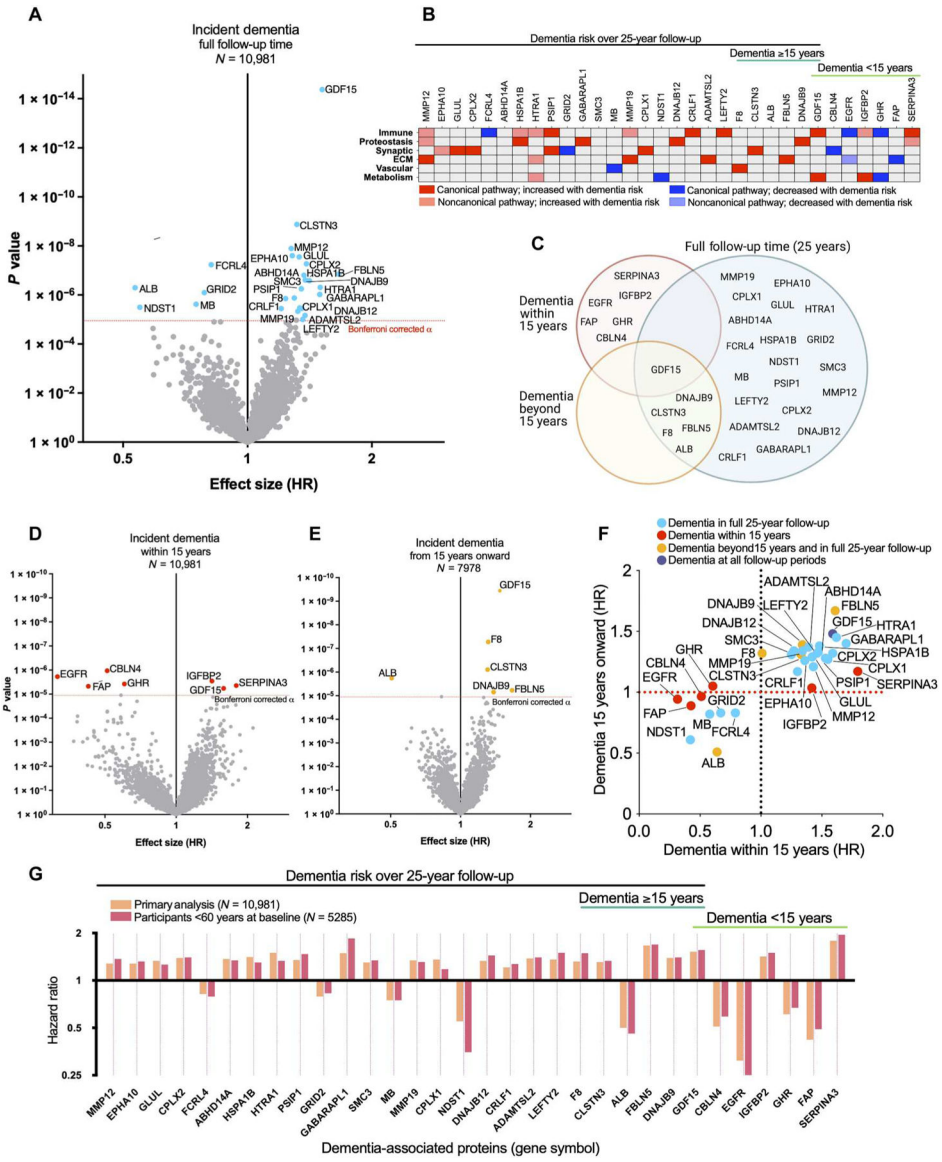

Fig. 1. Proteome-wide association study for 25-year dementia risk.

Hazard ratios (HRs) for all analyses were derived from Cox proportional hazards regression models adjusted for age, sex, race-study center, education, *APOE*ε4 status, and estimated glomerular filtration rate (eGFR) creatinine, body mass index, diabetes, hypertension, and smoking status at the time of protein assessment. (**A**) Volcano plot displays the HRs (*x* axis) and two-sided *P* values (*y* axis) for the association of $\log_2$ protein abundance with incident dementia. Proteins above the horizontal red line maintained a significant association after Bonferroni correction. (**B**) The majority of dementia-associated proteins were implicated in one of six biological pathways based on associated Gene Ontology terms. (**C**) Venn diagram shows candidate dementia-associated proteins from analysis of full-term, near-term, and long-term dementia risk. (**D**) Volcano plot displays the association of $\log_2$ protein abundance with incident dementia occurring within 15 years of follow-up (near-term dementia). (**E**) Volcano plot displays the association of $\log_2$ protein abundance with incident

dementia occurring beyond 15 years of follow-up (long-term dementia). (**F**) HRs for all 32 dementia-associated proteins in an analysis of near-term dementia risk (*x* axis) plotted against HRs from an analysis of long-term dementia risk (*y* axis). Color indicates in which analyses proteins were found to be statistically significant at a proteome-wide significance threshold. (**G**) This figure compares HRs from the primary analyses with HRs derived from participants below age 60 at the time blood was drawn for protein measurement. To make HRs directly comparable to HRs derived from the primary analysis, the six proteins associated with near-term dementia risk were examined in relation to dementia occurring within 15 years (n = 5285; 66 dementia cases). All other proteins were examined using the full follow-up time (n = 5285; 525 dementia cases).

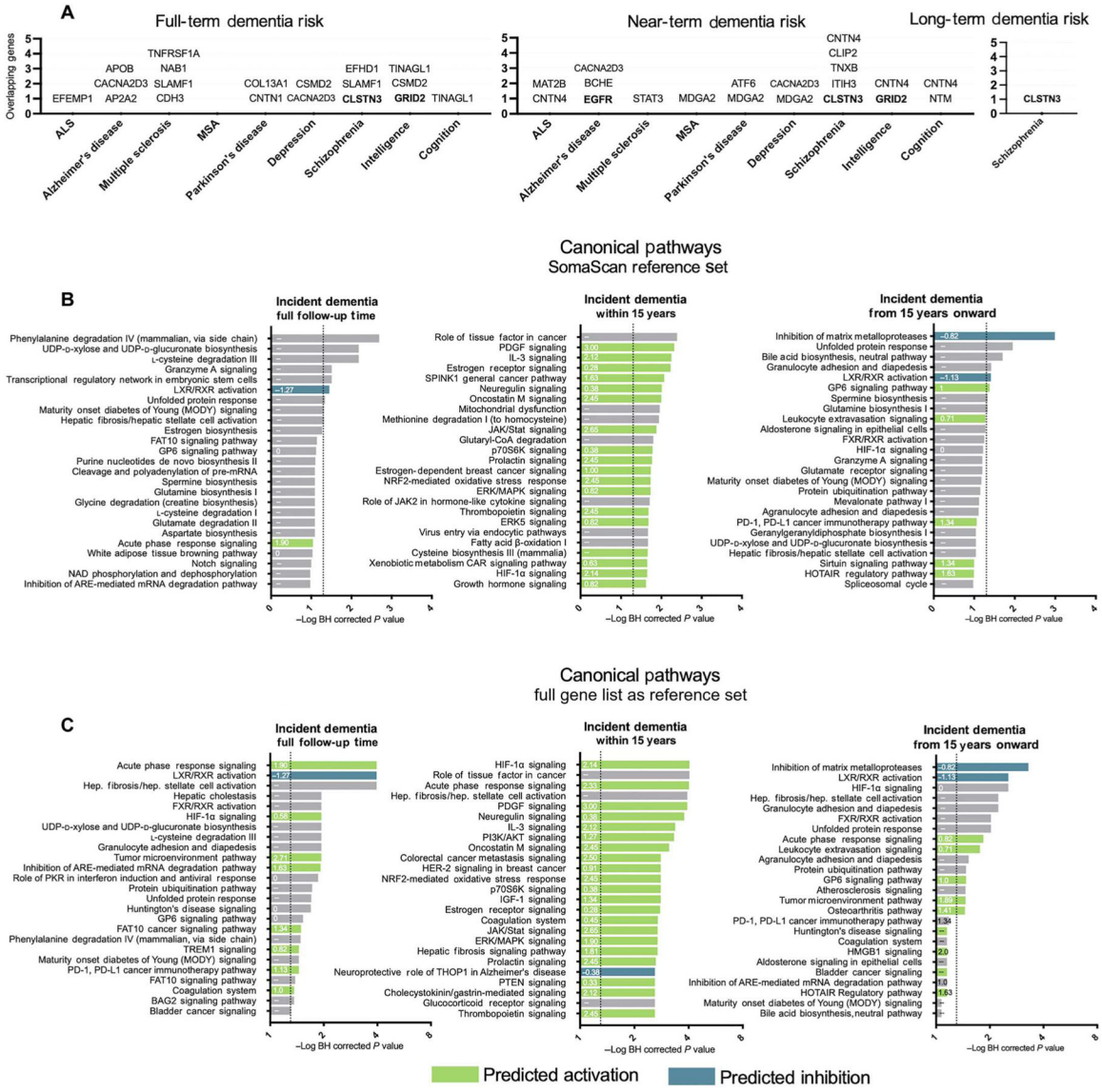

Fig. 2. Protein-neurologic disease/trait gene overlap and enriched biological pathways for dementia-associated proteins.

(**A**) Proteins significantly associated with dementia risk (FDR-corrected $P < 0.05$) and coded for by genes linked to GWAS risk variants for neurodegenerative and psychiatric disease, intelligence, and cognition. Gene lists were based on GWAS catalog summary statistics [by Yang and colleagues (49)] and recent AD GWAS (14). Bolded proteins were associated with dementia risk at a Bonferroni-corrected threshold. (**B**) Canonical (biological) pathways were identified using Ingenuity Pathway Analysis (IPA). The top 25 pathways for each analysis are displayed. Statistical significance was defined as an FDR-corrected $P < 0.05$ (one-sided) using right-tailed Fisher's exact test. The threshold for statistical significance is represented by the vertical dotted line. Number in each bar is a Z-score which indicates the predicted degree of pathway activation or inhibition. The direction of activation could not be predicted for gray bars. The extent of activation could not be predicted for bars with no Z-scores. Results presented in the top row are derived using

the full set of SomaScan proteins included in the study as a reference gene set. PDGF, platelet-derived growth factor; HIF-1α, hypoxia-inducible factor–1α. (**C**) Results presented in the second row are derived using the full gene list in the IPA database as the reference gene set. AKT, protein kinase B; ALS, amyotrophic lateral sclerosis; ARE, AU-rich element; BAG2, bcl2-associated athanogene 2; CAR, chimeric antigen receptor; GP6, glycoprotein VI; HER2, human epidermal growth factor receptor 2; HMGB1, high-mobility group box 1; IGF1, insulin-like growth factor 1; LXR/RXR, liver X receptor/retinoid X receptor; MSA, multiple system atrophy; NAD, nicotinamide adenine dinucleotide; Nrf2, nuclear factor-erythroid factor 2-relaed factor 2; PD-1, programmed death receptor-1; PD-L1, programmed death receptor-1 ligand; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; THOP1, thimet oligopeptidase 1.

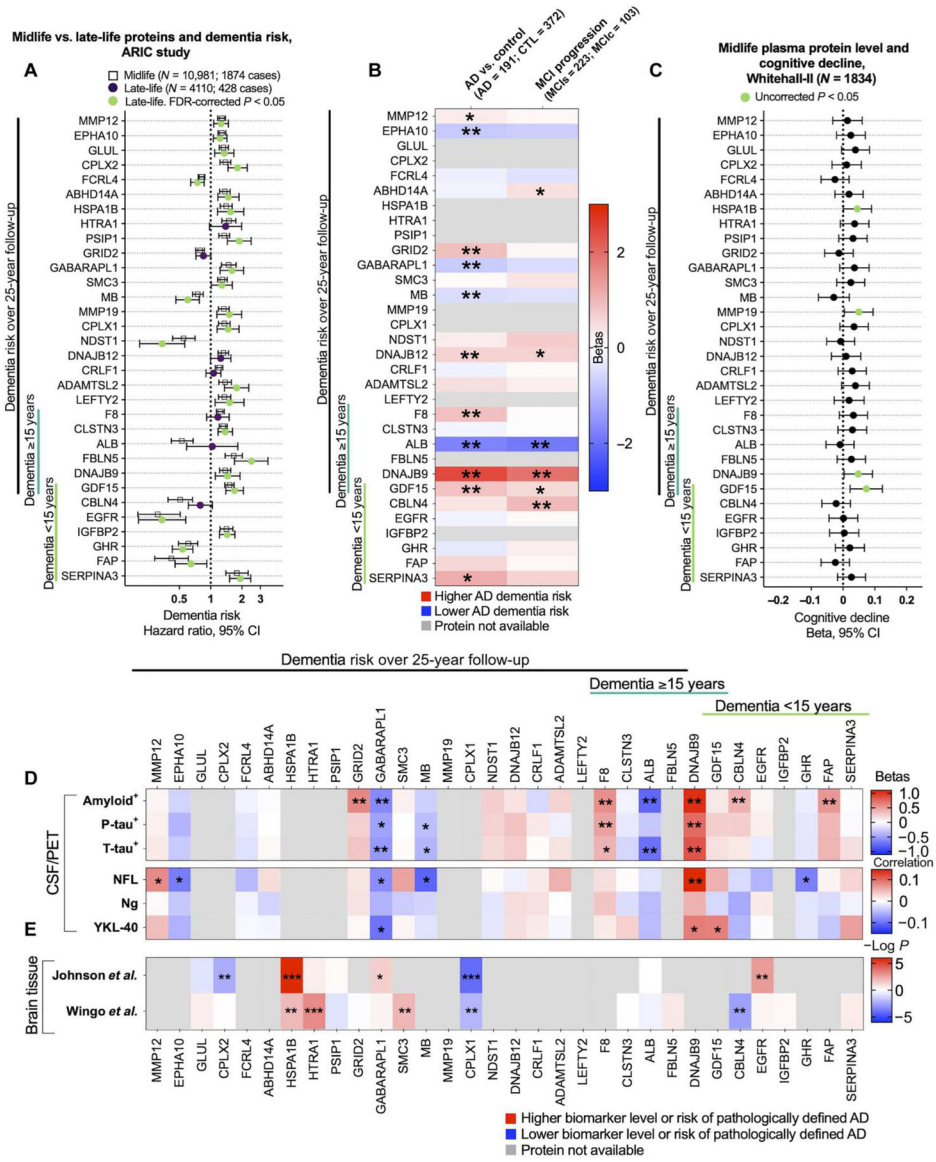

Fig. 3. Dementia-associated proteins are associated with Alzheimer's dementia, neuropathological changes, and CSF biomarkers.

(**A**) Hazard ratios (HRs) from a Cox proportional hazards model relating proteins measured in the ARIC late-life cohort to 5-year dementia risk (n = 4110; 428 cases). Late-life HRs (circles) are plotted next to midlife HRs (box) for the same protein derived from the ARIC discovery analysis. All models are adjusted for age, sex, race-study center, education, *APOE*ϵ4 status, estimated glomerular filtration rate (eGFR) creatinine, body mass index, diabetes, hypertension, and smoking status at the time of protein assessment. (**B**) Beta coefficients for a cross-sectional association of candidate proteins with clinically defined Alzheimer's disease (AD) (versus cognitively unimpaired status) and progression to AD (versus cognitively stable) among participants with mild cognitive impairment (MCI) in the EMIF-AD study derived using logistic regression. **Statistically significant (two-tailed P < 0.05) after FDR correction. *Statistically significant based on uncorrected two-tailed P < 0.05. (**C**) Beta coefficients for the association of candidate proteins with 20-year cognitive

decline in the Whitehall II study derived using linear regression adjusted for age, sex, ethnicity, *APOE*ϵ4 status, and eGFR. Higher values indicate elevated proteins abundance is associated with greater cognitive decline. (**D**) Cross-sectional association between candidate plasma proteins and CSF biomarkers in the EMIF-AD study. **Statistically significant (two-tailed $P < 0.05$) after FDR correction. *Statistically significant based on uncorrected two-tailed $P < 0.05$. Sample sizes: amyloid, $n = 972$; P-tau, $n = 876$; T-tau, $n = 880$; NFL, $n = 643$; Ng, $n = 598$; YKL-40, $n = 649$. (**E**) Results from a brain proteomic study of AD for candidate proteins. Results derived from Johnson *et al.* (18) and Wingo *et al.* (19). Heatmap displays signed *P* values. *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$. MCIc, mild cognitive impairment converter; MCIs, mild cognitive impairment stable; NFL, neurofilament light chain; Ng, neurogranin; p-tau181, phosphorylated tau181; T-tau, total tau; YKL-40, chitinase-3-like protein 1 (CHI3L1).

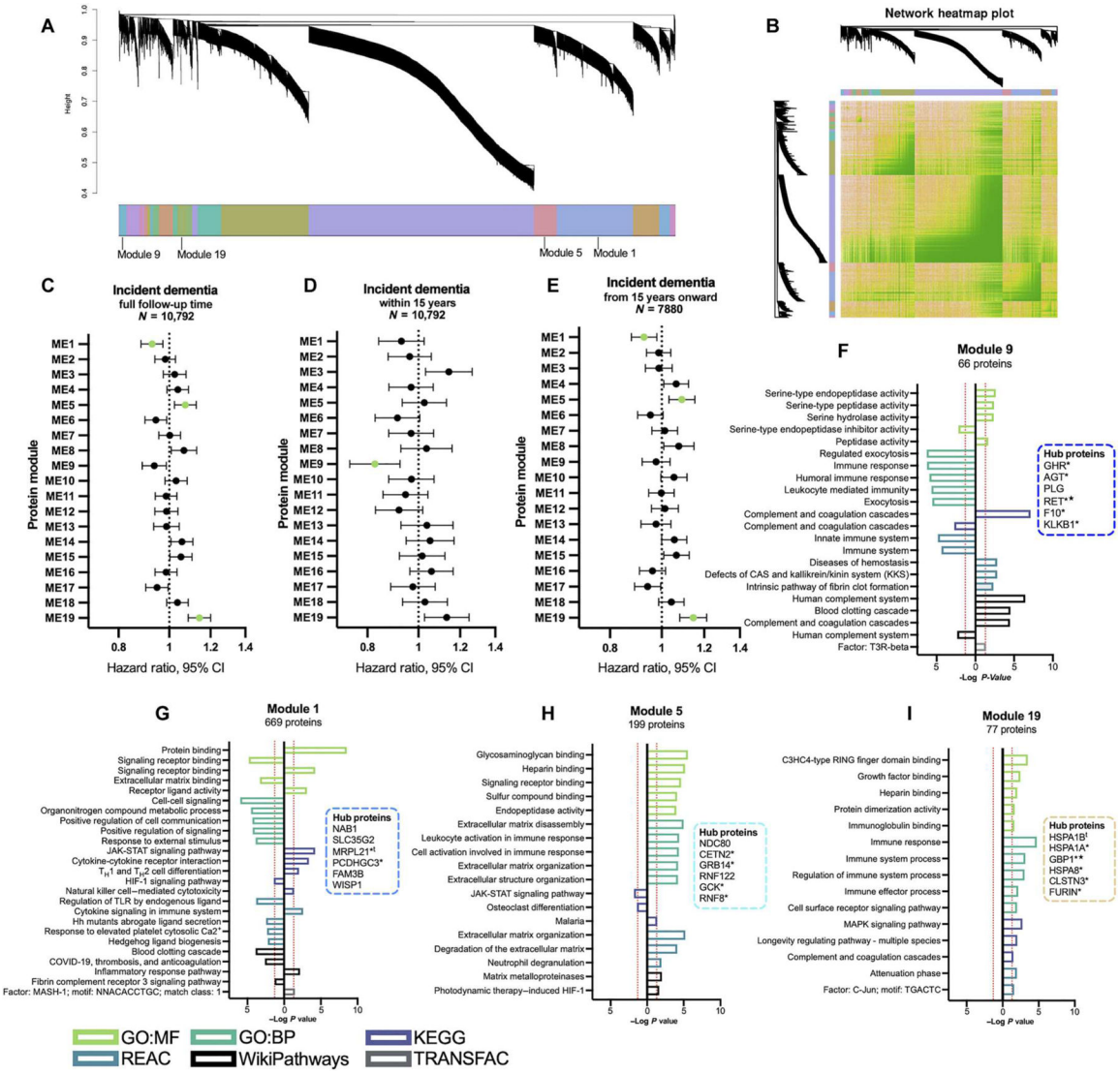

Fig. 4. Midlife protein networks are associated with near-term and long-term dementia risk.

(**A**) Hierarchical cluster tree of 4877 proteins measured at baseline visit of the ARIC discovery analysis (visit 3; 1993–95). The band displays the separation of proteins into 19 modules using Netboost clustering. (**B**) The Topological Overlap Matrix displayed as a heatmap for visualization of protein networks. Darker green represents higher protein-protein adjacency. (**C**) Association of module expression (module eigenprotein) with 25-year dementia risk. (**D**) Association of module expression with near-term dementia risk (dementia within 15 years). (**E**) Association of module expression with long-term dementia risk (dementia occurring after 15 years). Hazard ratios (HRs) represent the adjusted dementia risk per standard deviation increase in module expression. (**F** to **I**) Enrichment analysis results for the proteins of modules 9, 1, 5, and 19, respectively. Top five significantly enriched pathways ($P < 0.05$) from each database are displayed. P value are corrected for multiple comparisons using the g:SCS algorithm in g:Profiler. Left-facing bars display pathway enrichment for proteins negatively associated with module expression. Right-facing bars display pathway enrichment for proteins positively associated with module expression.

We display the six proteins in each network most highly correlated with overall network expression (hub proteins). * indicates gene encoding for hub protein is differentially expressed in AD brains as identified using the AMP-AD Sage Bionetworks Agora platform. ★Protein has been nominated as an AD therapeutic target by AMP-AD.

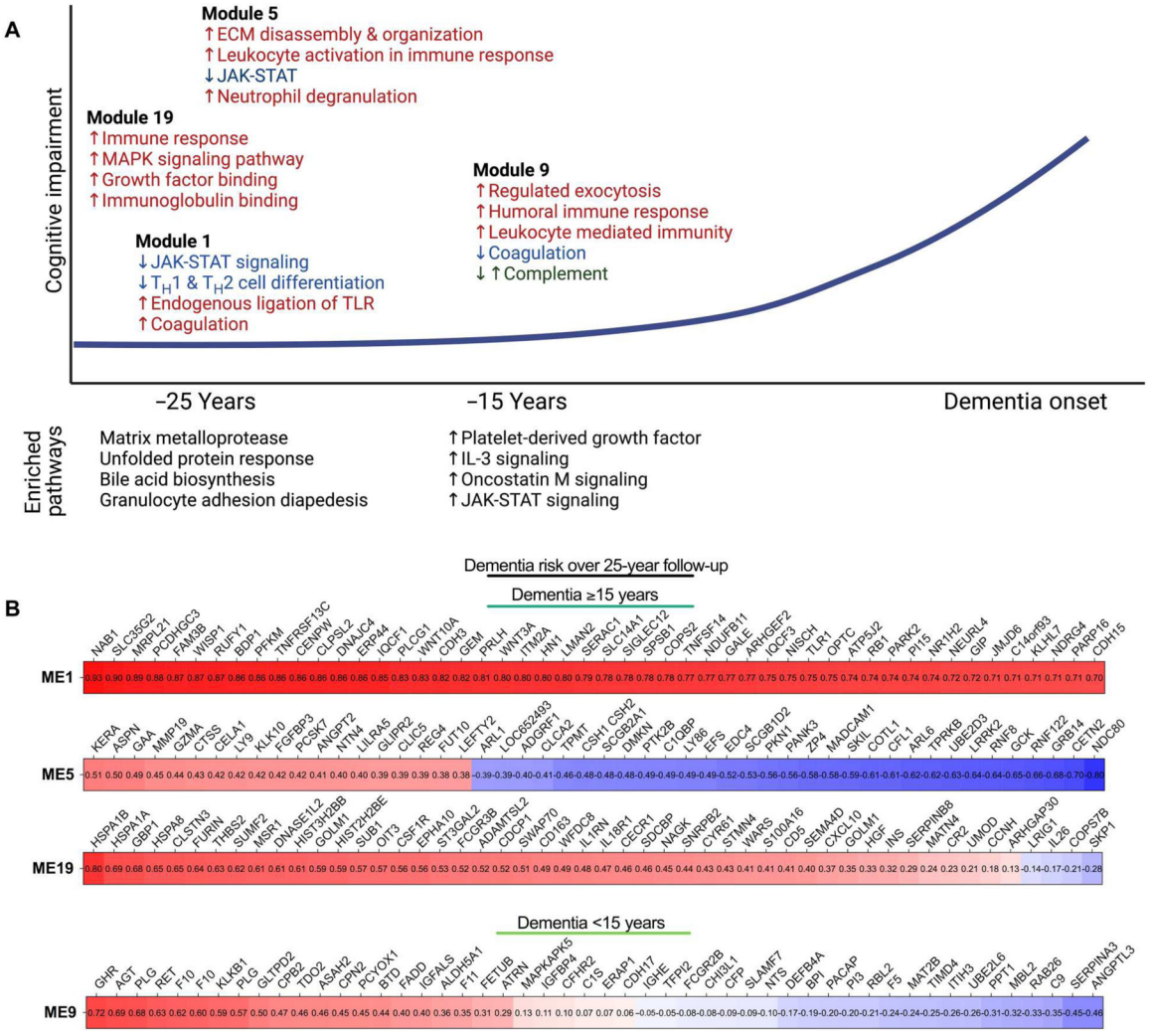

Fig. 5. Dementia-associated protein modules and enriched pathways in the decades before dementia onset.

(**A**) The hypothesized temporal sequence of dementia-associated protein modules and enriched biological (canonical) pathways over the 25-year follow-up period. (**B**) Top 50 proteins for each dementia-associated protein module based on module membership. Module membership is defined as the correlation between protein abundance and overall module expression [module eigenprotein (ME)]. Module membership values are provided in each cell.

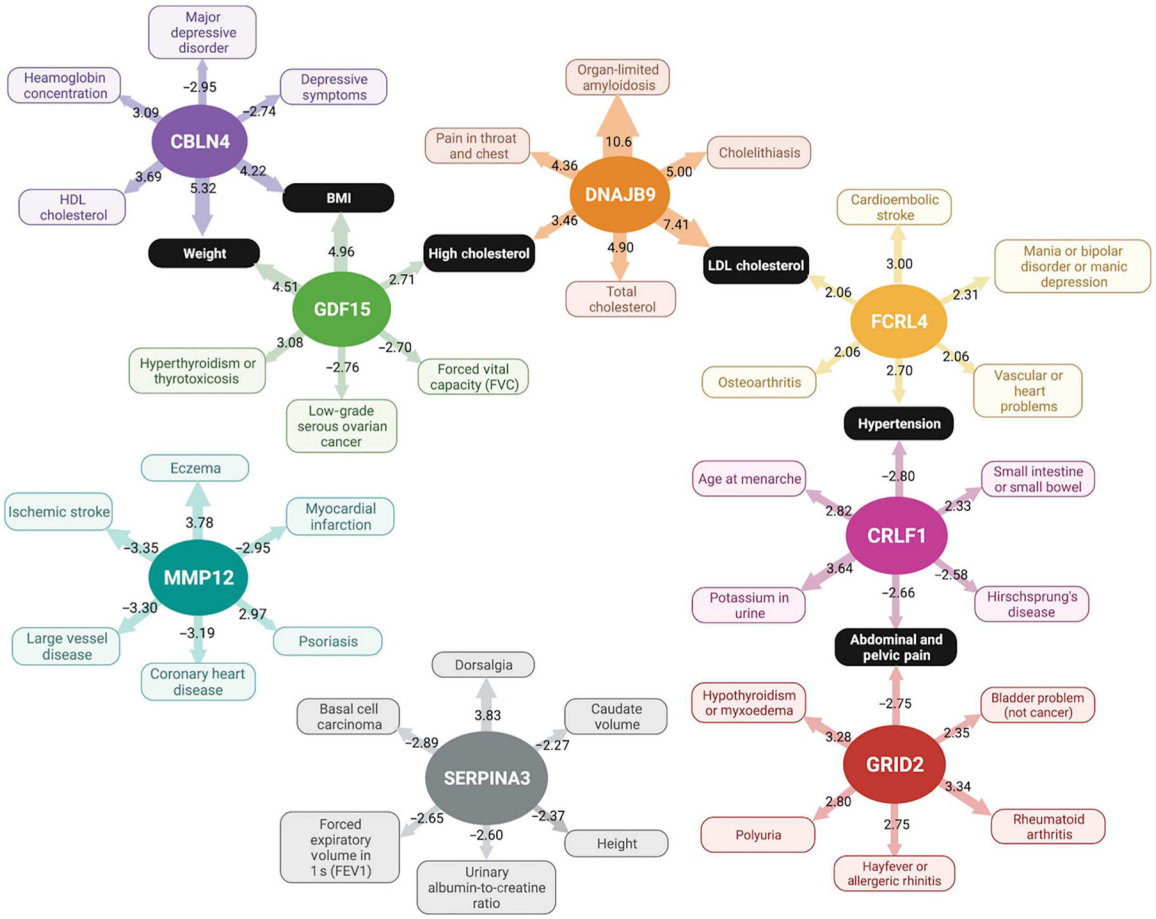

Fig. 6. Potential causal relationships between dementia-associated proteins and non-AD phenotypes.

Figure displays Mendelian randomization results derived from the Proteome PheWAS browser (https://epigraphdb.org/pqtl) published by Zheng *et al.* (27). Of the 32 dementia-associated plasma proteins identified in the present study, 9 were examined in this PheWAS study. All phenotypes displayed above were significantly associated with plasma protein level ($P < 0.05$) in a Mendelian-randomization analysis conducted using Wald ratio or inverse variance weighted (IVW) methods. The thickness of the arrow and associated values represents the effect size of the protein exposure on the phenotype divided by the corresponding standard error (Z-statistic). The graph displays the top six phenotypes most strongly associated with each plasma protein. The full list of phenotypes associated with each plasma protein is provided as supplementary data.

Table 1.

Examination of Alzheimer's disease potential causal effects for the dementia-associated proteins using bidirectional two-sample Mendelian randomization.

The ARIC study was used for the GWAS of plasma protein abundance and protein network expression. Alzheimer's disease-associated single nucleotide polymorphisms (SNPs) with reported pleiotropic association with potential confounders (immune cell counts, cholesterol, C-reactive protein abudance, etc.) are excluded.

Gene name	Outcome trait	Inverse variance weighting (IVW) estimate					
		Forward			Backward		
		No. of SNPs	Slope ± SE	*P* value	No. of SNPs	Slope ± SE	*P* value
ABHD14A	AD	3[*]	−0.207 ± 0.417	0.619	28[†]	0.023 ± 0.006	4.89×10^{-4}[‡]
ADAMTSL2	AD	3	0.099 ± 0.247	0.687	28[†]	−0.005 ± 0.006	0.480
ALB	AD	1	−0.038 ± 0.376	0.919	29[†]	−0.004 ± 0.003	0.239
CBLN4	AD	6[*]	−0.228 ± 0.143	0.110	30[†]	0.001 ± 0.006	0.930
CLSTN3	AD	3	−0.410 ± 0.200	4.02×10^{-2}	26[†]	−0.023 ± 0.011	4.28×10^{-2}
CPLX1	AD		No instruments identified		30[†]	0.001 ± 0.005	0.809
CPLX2	AD		No instruments identified		29[†]	0.024 ± 0.007	3.04×10^{-4}[‡]
CRLF1	AD	3	0.147 ± 0.159	0.353	30[†]	−0.006 ± 0.009	0.482
DNAJB12	AD	4	0.244 ± 0.347	0.481	27[†]	0.002 ± 0.006	0.798
DNAJB9	AD	4	−0.285 ± 0.153	0.063	30[†]	−0.002 ± 0.007	0.812
EGFR	AD	3	0.466 ± 0.376	0.215	30[†]	0.004 ± 0.004	0.279
EPHA10	AD	3	0.202 ± 0.182	0.269	29[†]	−0.004 ± 0.008	0.651
F8	AD	9	−0.067 ± 0.059	0.253	26[†]	0.006 ± 0.009	0.492
FAP	AD	2[*]	−0.314 ± 0.359	0.382	30[†]	−0.003 ± 0.005	0.582
FBLN5	AD		No instruments identified		28[†]	0.007 ± 0.004	0.096
FCRL4	AD	18	−0.010 ± 0.023	0.669	30[†]	−0.010 ± 0.012	0.403
GABARAPL1	AD	4[*]	0.046 ± 0.444	0.917	27[†]	0.004 ± 0.004	0.392
GDF15	AD	7[*†]	0.133 ± 0.083	0.110	30[†]	0.018 ± 0.008	3.55×10^{-2}
GHR	AD	2[*]	−0.045 ± 0.425	0.917	29[†]	−0.031 ± 0.009	4.96×10^{-4}[‡]

Gene name	Outcome trait	Inverse variance weighting (IVW) estimate					
		Forward			Backward		
		No. of SNPs	Slope ± SE	*P* value	No. of SNPs	Slope ± SE	*P* value
GLUL	AD	1	−0.291 ± 0.315	0.356	30†	−0.007 ± 0.008	0.366
GRID2	AD	2	−0.076 ± 0.186	0.683	30†	−0.012 ± 0.009	0.191
HSPA1B	AD	1	−0.361 ± 0.357	0.313	30†	0.001 ± 0.006	0.806
HTRA1	AD	1	−0.138 ± 0.468	0.767	31	−0.011 ± 0.006	0.056
IGFBP2	AD		No instruments identified		31	0.030 ± 0.012	1.36×10^{-2}
LEFTY2	AD	3	−0.058 ± 0.165	0.725	26†	0.011 ± 0.006	0.062
MB	AD	1	−0.098 ± 0.334	0.769	29†	0.004 ± 0.008	0.656
MMP12	AD	9*	−0.015 ± 0.054	0.780	31	0.028 ± 0.010	6.44×10^{-3}
MMP19	AD	2	0.053 ± 0.293	0.856	31	0.009 ± 0.006	0.145
NDST1	AD	7*	−0.282 ± 0.250	0.259	30†	0.009 ± 0.003	4.84×10^{-3}
PSIP1	AD	5	−0.177 ± 0.097	0.068	29†	0.018 ± 0.007	1.11×10^{-2}
SERPINA3	AD	4	−0.259 ± 0.116	2.63×10^{-2}	30†	0.00012 ± 0.007	0.986
SMC3	AD	2	0.370 ± 0.286	0.196	30†	0.013 ± 0.007	0.072
Module 1	AD	6	−0.150 ± 8.193	0.985	29†	−0.0001 ± 0.0002	0.367
Module 5	AD	6	−0.364 ± 6.823	0.958	27†	0.00005 ± 0.0002	0.752
Module 9	AD	3	1.847 ± 7.875	0.815	30†	0.0002 ± 0.0002	0.274
Module 19	AD	2	−12.045 ± 11.153	0.280	30†	−0.0001 ± 0.0002	0.639

*Protein quantitative trait loci (pQTL) identified in INTERVAL are added.

†Pleiotropy or heterogeneity outlier was detected. IVW analysis was reperformed excluding the outlier identified by the RadialMR method.

‡Significant causal association at Bonferroni-adjusted significance threshold (0.05 per number of tested associations): 1.56×10^{-3} for analyses in the forward direction; 1.39×10^{-3} for analyses in the backward direction.

Sci Transl Med. Author manuscript; available in PMC 2024 July 19.