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RESEARCH ARTICLE

Proteome-wide Analyses Identified Cortical Proteins Associated With Resilience for Varied Cognitive Abilities

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Abstract

Background and Objectives

Prior work suggests that cognitive resilience may contribute to the heterogeneity of cognitive decline. This study examined whether distinct cortical proteins provide resilience for different cognitive abilities.

Methods

Participants were from the Religious Orders Study or the Rush Memory and Aging Project who had undergone annual assessments of 5 cognitive abilities and postmortem assessment of 9 Alzheimer disease and related dementia (ADRD) pathologies. Proteome-wide examination of the dorsolateral prefrontal cortex using tandem mass tag and liquid chromatography-mass spectrometry yielded 8,425 high-abundance proteins. We applied linear mixed-effect models to quantify residual cognitive change (cognitive resilience) of 5 cognitive abilities by regressing out cognitive decline related to age, sex, education, and indices of ADRD pathologies. Then we added terms for each of the individual proteins to identify cognitive resilience proteins associated with the different cognitive abilities.

Results

We included 604 decedents (69% female; mean age at death = 89 years) with proteomic data. A total of 47 cortical proteins that provide cognitive resilience were identified: 22 were associated with specific cognitive abilities, and 25 were common to at least 2 cognitive abilities. NRN1 was the only protein that was associated with more than 2 cognitive abilities (semantic memory: estimate = 0.020, SE = 0.004, $p = 2.2 \times 10^{-6}$; episodic memory: estimate = 0.029, SE = 0.004, $p = 5.8 \times 10^{-1}$; and working memory: estimate = 0.021, SE = 0.004, $p = 1.2 \times 10^{-7}$). Exploratory gene ontology analysis suggested that among top molecular pathways, mitochondrial translation was a molecular mechanism providing resilience in episodic memory, while nuclear-transcribed messenger RNA catabolic processes provided resilience in working memory.

Discussion

This study identified cortical proteins associated with various cognitive abilities. Differential associations across abilities may reflect distinct underlying biological pathways. These data provide potential high-value targets for further mechanistic and drug discovery studies to develop targeted treatments to prevent loss of cognition.

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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

AD = Alzheimer disease; **ADRD** = Alzheimer disease and related dementias; **DLPC** = dorsal lateral prefrontal cortex; **FDR** = false discovery rate; **GO** = gene ontology; **MAP** = Rush Memory and Aging Project; **MCI** = mild cognitive impairment; **mRNA** = messenger RNA; **ROS** = Religious Orders Study; **TMT** = tandem mass tag.

Introduction

Accumulating evidence has highlighted the heterogeneity of cognitive decline in older adults. Elucidating the biology underlying this heterogeneity is crucial for identifying the mechanisms and targeted treatments that can prevent or decelerate late-life cognitive impairment. Alzheimer disease and related dementia (ADRD) pathology is a major driver of later-life cognitive heterogeneity. After regressing out the negative cognitive effects of ADRD pathologies, we are left with a wide range of person-specific cognitive decline. The average person is defined as having average resilience, and those declining slower and faster than average have more or less resilience. This approach allowed us to identify biologic mechanisms, such as genes, proteins, and glycoproteoforms, which are associated with higher or lower cognitive resilience after we regress out ADRD pathologies, that is, residual cognitive change.[1-9] In particular, we previously reported that a group of cortical proteins are associated with a global summary measure of cognitive ability.[10] The current work extends prior work and examines to what extent different cognitive abilities are associated with shared and specific resilience proteins.

Cognition is a complex phenotype composed of diverse interrelated networks that underlie varied abilities relating to the selection, storage, manipulation, and organization of information.[11-13] Performance on such varied abilities requires effortful cognitive processing, which may take on various forms and may likewise decline nonuniformly.[14] Evidence suggests that different cognitive abilities may differ in their associations with ADRD pathologies and molecular mechanisms driving aging and disease.[15-18] Our prior studies interrogated a composite measure of global cognition constructed from diverse cognitive abilities,[1-3] so the specificity of resilience proteins for different cognitive abilities is unknown. Furthermore, additional proteins may be associated with specific cognitive domains and be missed with a measure of global cognition.

To fill this knowledge gap, this study was designed to identify the associations of cognitive resilience proteins and their specificity for 5 cognitive abilities. We analyzed clinical and postmortem data available from 600 decedents participating in 2 community-based cohort studies that obtained repeated annual assessments of 19 cognitive tests to evaluate the following: (1) episodic memory, (2) semantic memory, (3) working memory, (4) perceptual speed, and (5) visuospatial ability and postmortem collection of indices of 9 ADRD brain pathologies and proteome-wide data collected from dorsal lateral prefrontal cortex (DLPFC).

Methods

Study Participants

Participants were community-dwelling older adults enrolled in 1 of 2 ongoing cohort studies of aging and dementia, the Religious Orders Study (ROS)[19] and the Rush Memory and Aging Project (MAP).[20] Participants without known dementia who agreed to annual clinical testing and brain donation during death were enrolled in these studies. The ROS began enrollment on January 1, 1994, and the MAP began enrollment on September 1, 1997. The ROS and MAP are conducted by the same team of investigators and share a large common core of harmonized clinical and postmortem data collection that allows for these joint analyses. Both studies were approved by a Rush University Medical Center Institutional Review Board. At enrollment, each participant provided written informed consent and signed the Uniform Anatomical Gift Act.

Through October 23, 2019, 1,654 ROS and MAP participants had died and undergone brain autopsies (autopsy rate >85%). This study focused on a subset of 604 decedents who had tandem mass tag (TMT) proteomics analyses performed using frozen tissue samples obtained from the DLPFC; these participants were selected as part of a convenience sample. TMT-mass spectrometry proteomic data are ongoing. In prior work, we analyzed data on the first installment that included 400 participants[2]; since then, proteomic data on an additional 204 participants were obtained.

Assessment of Cognitive Function

Cognitive Abilities

Detailed neuropsychological assessment was administered at annual intervals with a battery of performance tests, as previously described.[21] For these analyses, we used previously established summary measures of episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability. Raw scores on individual tests were converted to z scores using the baseline mean and SD of the entire cohort, and the z scores of component tests were averaged to yield composite scores, as previously described.[15]

Global Cognitive Function

A global composite score was derived by standardizing 19 tests using baseline mean and SDs of both cohorts. Repeated cognitive measures were used to estimate the rate of cognitive decline before death.

Assessment of Cognitive Status and Diagnosis

A 3-step process based on algorithms and clinical judgment was applied to diagnose Alzheimer and other dementias, as previously described.[20,22,23] Persons who did not meet the criteria for dementia but who show evidence of impairment in at least 1 cognitive domain are classified as mild cognitive impairment (MCI).

Assessment of Clinical Covariates

Demographic measures, such as age, sex, and number of years in formal education, are recorded at enrollment, and age of death is calculated using self-reported date of birth and date of death and autopsy.[24]

Assessment of ADRD Pathologies

Upon death, the brain was removed and hemisected following standard procedure.[25] One hemisphere was prepared for histologic evaluation and the other hemisphere was frozen for the collection of omics. The fresh slabs were fixed in 4% paraformaldehyde. The hemisphere was cut into 1-cm coronal slabs. Tissue blocks from predetermined regions were dissected, embedded in paraffin, and cut into 6 and 20 micro sections.[25] Measured indices of ADRD pathologies included (1) Alzheimer disease (AD) pathology, (2) hippocampal sclerosis, (3) TAR DNA-binding protein 43, (4) Lewy bodies, (5) macroinfarcts, (6) microinfarcts, (7) cerebral angiopathy, (8) atherosclerosis, and (9) arteriolosclerosis.[26] Neuropathologic data collection and assessment were performed blinded to all clinical and cognitive data, as previously described.[27]

Assessment of the Proteome

Mass spectrometry–based proteomics using isobaric TMTs was conducted on frozen tissue samples of the DLPFC. In brief, the samples were homogenized, and the protein concentration was determined. After protein digestion, isobaric TMT peptide labeling and high pH fractionation were performed. Fractions were then analyzed by liquid chromatography-mass spectrometry. The resulting mass spectrometry spectra were searched against the UniProt human protein database, with individual protein abundance checked against the global internal standard. Protein sequencing batch, postmortem interval, and donor age were estimated and removed using linear regression. Additional details about TMT proteomics and quality control are provided in prior publications.[28]

Statistical Analysis

We first describe the characteristics of the participants, which include demographic characteristics, scores on each of the cognitive abilities at baseline and proximate to death, proportions of diagnoses of MCI and AD proximate to death, and the frequencies of 9 ADRD pathologies. We also estimate rates of cognitive decline in each of the 5 cognitive measures, and global cognition, using linear mixed-effects models.

Cognitive Decline, Residual Cognitive Change, and Cognitive Resilience

To identify proteins that are associated with cognitive resilience, we first ran linear mixed-effects models using each of the 5 cognitive abilities as longitudinal continuous outcomes and adjusting for age at death, sex, and education to investigate the annual rate of cognitive decline (model 0). To compare our results with those of prior studies, we also repeated our analyses with global cognition. We then ran the 6 separate proteome-wide studies by adjusting for demographics and 9 ADRD pathologies (model 1). Proteins that were associated with residual cognitive change were termed cognitive resilience proteins. To minimize false positives due to multiple testing, statistical significance was determined at an a priori alpha level of 5×10^{-6}, which corresponds to a Bonferroni correction for 10,000 tests, as previously performed.[1,2,10]

To calculate the amount of variance explained by the identified cognitive resilience proteins, we first calculated the variance accounted for by a model, which included terms for age before death, sex, and education (model 0). Then we added 9 neuropathologic indices (model 1) to be able to compare model 1 with model 0 and determine additional variance explained by ADRD pathologies. Last, we added the respective resilience proteins for each outcome in the model (model 2). We compared the variance accounted by the terms in model 2 and model 1. The variance difference between these models represents the additional variance accounted for by the identified proteins over and above the variance due to demographics and 9 neuropathologies.

Gene Ontology

Gene ontology (GO) enrichment analysis was performed on the proteins associated with cognitive resilience to identify underlying biological pathways associated with resilience proteins and to determine whether there is evidence for differential associations between cognitive abilities and biological pathways.

The analyses were conducted using SAS/STAT software, version 9.4 (SAS Institute Inc., Cary, NC) and R software version 4.2.2.[29] GO analysis was conducted using Functional Mapping and Annotation of Genome-Wide Association Studies with FUMA.[30] GO terms were clustered (hierarchical clustering, Ward linkage) into groups of related terms using Resnik semantic similarity measure implemented in the R package GOSemSim.[31]

Standard Protocol Approvals, Registrations, and Patient Consents

Both studies were approved by an Institutional Review Board of Rush University Medical Center. Written informed consent was obtained from all study participants as was an Anatomical Gift Act for organ donation.

Data Availability

All data included in these analyses are available through the Rush Alzheimer's Disease Center Research Resource Sharing Hub, which can be found at radc.rush.edu. It has

descriptions of the studies and available data. Investigators can create an account and submit requests for deidentified data.

Results

Characteristics of the Analytic Cohort

In total, 8,425 proteins in 604 persons (69% female; mean age at death = 89 years) passed quality control and were thus analyzed in this study. The demographic characteristics of the subset of participants with TMT proteomic data and the overall ROS and MAP autopsied participants were similar; however, in general, participants with TMT data had fewer neuropathologic conditions.[2] Their clinical and postmortem characteristics are detailed in Table 1. At baseline, the mean global cognition was −0.003 standard units (SD = 0.5). The range of cognitive function for the specific abilities at baseline varied with perceptual speed showing the lowest mean scores (mean = −0.075; SD = 0.8) and visuospatial ability showing the highest (mean = 0.084, SD = 0.7). At the last visit proximate to death, all abilities had lower mean scores, with perceptual speed showing again the lowest mean standardized score (mean = −1.01; SD = 1.0), while visuospatial ability maintained the highest, among the cognitive abilities (mean = −0.45; SD = 1.0). At their last visit before death, more than one-half of participants had normal cognition, more than a quarter of participants had MCI, and less than another quarter were diagnosed with AD dementia.

For descriptive purposes, we dichotomized the 9 ADRD pathologies. The most common pathology was AD pathology, with 62.1% of participants having a pathologic AD diagnosis based on a modified National Institute on Aging and Alzheimer's Association criteria (Table 1). Approximately 8% (n = 47) of participants did not have any of the 9 measured ADRD pathologies. The median number of ADRD pathologies was 3 (interquartile range = 2–4), with 16% of participants (n = 96) having 1 pathology, 23% (n = 136) having 2 pathologies, 21% (n = 127) having 3 pathologies, 18% (n = 111) having 4 pathologies, 8% having 5 pathologies, 5% (n = 29) having 6 pathologies, and <2% (n = 11) having between 7 and 9 pathologies.

Identification of Cognitive Resilience Proteins

The rate of decline for the specific abilities was heterogeneous ranging from −0.13 standard units per year (SE = 0.01, $p < 0.001$) for perceptual speed to −0.06 standard units per year (SE = 0.05, $p < 0.001$) for visuospatial ability. Both semantic memory and working memory declined by −0.09 standard units per year (SE = 0.01, $p < 0.001$). Episodic memory and global cognition declined at a rate of −0.10 standard units per year (SE = 0.01, $p < 0.001$).

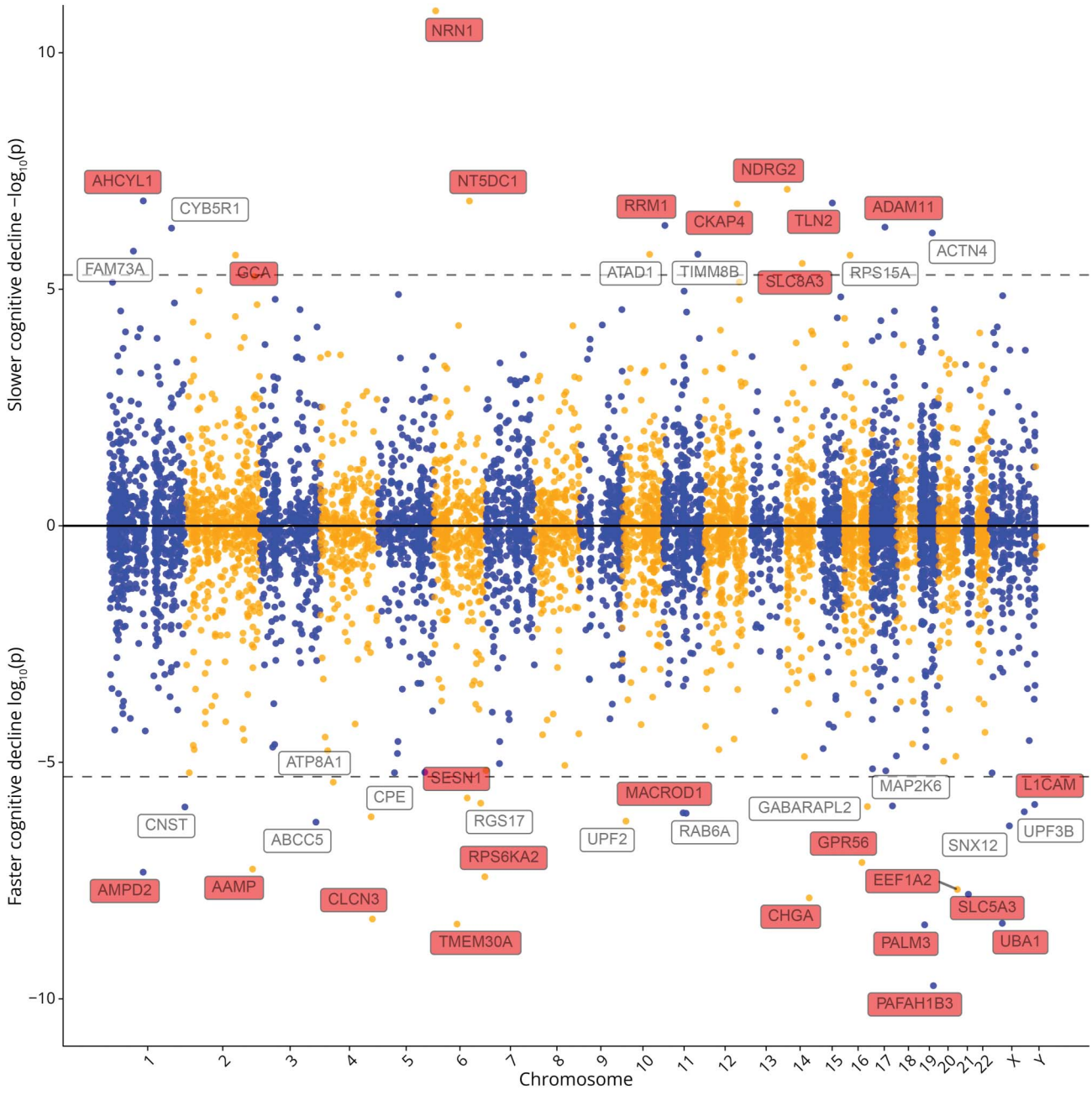
We first repeated our prior analysis with global cognition. With more than 50% greater sample size, we identified 42 proteins associated with global cognition, 34 more than previously reported. The magnitude and the direction of effect of the signal of all 8 proteins that were previously reported[2] were similar in this study. Using the reprocessed data with the updated sample size, all 8 proteins were replicated using false discovery rate (FDR) correction, while 3 (NRN1, ACTN4,

Table 1 Clinical and Postmortem Characteristics of the Analytic Cohort (N = 604)

Demographic measures	Mean (SD)/n (%)
Age at baseline, y, mean (SD)	81.2 (6.8)
Age at death, y, mean (SD)	89.7 (6.4)
Sex, female, n (%)	416 (69)
Education, y, mean (SD)	15.4 (3.4)
Clinical measures	
MMSE, mean (SD)	27.9 (2.1)
MCI at last visit, n (%)	169 (28)
AD dementia at last visit, n (%)	114 (24)
Cognitive measures at baseline and last visit, mean (SD)	
Global cognitive function	−0.003 (0.5), −0.785 (1.1)
Semantic memory	0.021 (0.6), −0.624 (1.2)
Episodic memory	−0.019 (0.7), −0.751 (1.3)
Working memory	0.056 (0.7), −0.596 (1.1)
Perceptual speed	−0.075 (0.8), −1.007 (1.0)
Visuospatial ability	0.084 (0.7), −0.450 (1.0)
Neuropathologic indices	
Postmortem interval, h, mean (SD)	8.1 (5.2)
Summary measure of AD pathology, mean (SD)	0.741 (0.4)
AD (NIA-AA criteria), n (%)	375 (62.1)
TDP-43, n (%)	176 (29.4)
Hippocampal sclerosis, n (%)	48 (8.0)
Lewy body, n (%)	151 (25.0)
Cerebrovascular pathologies	
Macroinfarcts, n (%)	48 (8.0)
Microinfarcts, n (%)	167 (27.7)
Atherosclerosis (moderate/severe), n (%)	194 (32.2)
Arteriolosclerosis (moderate/severe), n (%)	193 (32.1)
Cerebral amyloid angiopathy (moderate/severe), n (%)	183 (30.4)

Abbreviations: AD = Alzheimer disease; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; NIA-AA = National Institute on Aging and Alzheimer's Association; TDP-43 = TAR DNA-binding protein 43.

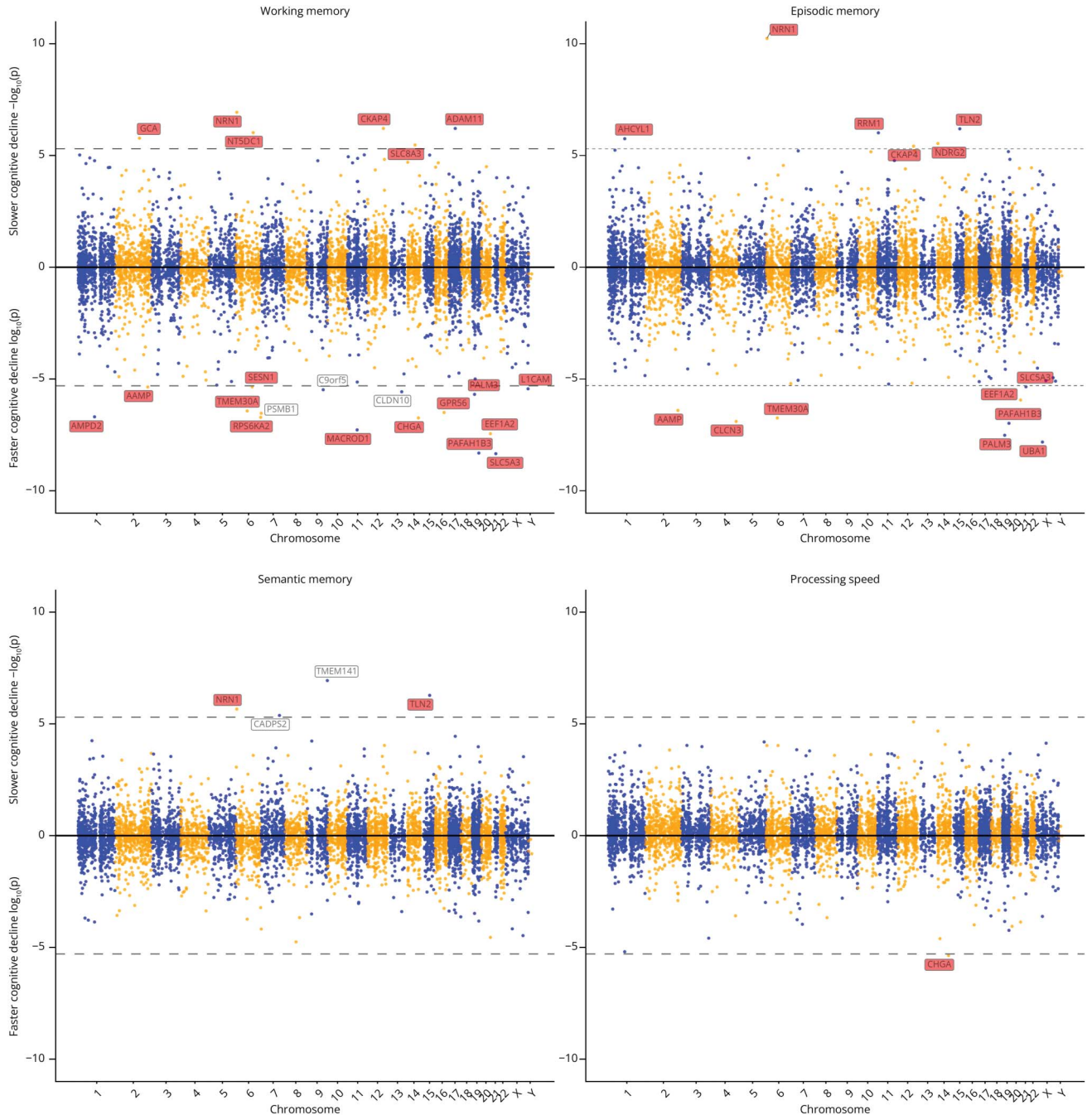
Figure 1 Proteome-wide Study of Global Cognition



Analyses are based on a summary measure representing global cognition and which is based on 5 cognitive outcomes (episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability). Each point on the plot represents the association of an individual protein with cognitive decline after controlling for age at death, sex, education, and 9 ADRD pathologies. NRN1 was the lead protein associated with cognitive resilience. The horizontal coordinate is the corresponding gene location within the chromosome (defined as the midpoint of the start and end positions). The vertical coordinate is the −log10 of the *p* value if the protein is associated with slower cognitive decline, that is, greater cognitive resilience, and log10 of the *p* value if it is associated with faster cognitive decline, that is, less resilience. The dashed lines correspond to the reference significance level representing $\alpha = 5 \times 10^{-6}$. The red boxes denote proteins that are associated with various cognitive abilities. The white boxes denote proteins that are specifically associated with global cognition. ADRD = Alzheimer disease and related dementias.

UBA1) made the Bonferroni cutoff and 5 (RPH3A, SGTB, CPLX1, SH3GL1, and EPHX4) remained nominally associated. The number of resilience proteins associated with the 5 cognitive domains was 22 for working memory, 14 for episodic memory, 4 for semantic memory, 1 for perceptual speed, and none for visuospatial ability. Chicago plots illustrate the associations of resilience proteins with global cognition (Figure 1) and each of the cognitive domains (Figure 2). The model results for all proteins associated with cognitive resilience for each of the cognitive abilities are included in eTable 1 (links.lww.com/WNL/D260). In total, we identified 47 unique proteins: 25 were associated with 1 or more of the cognitive outcomes, while 22 were specific to 1 cognitive outcome; specifically 17 were specific to global cognition and

Figure 2 Proteome-wide Study of Different Cognitive Abilities

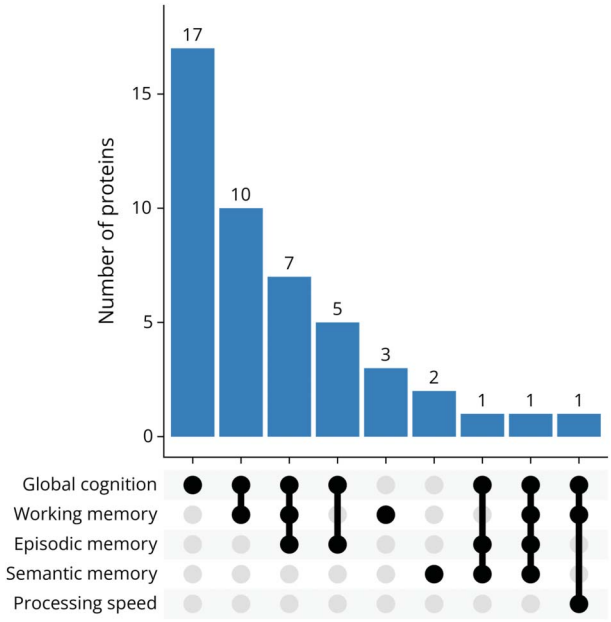


Analyses are based on the association of an individual protein with cognitive decline after controlling for age at death, sex, education, and 9 ADRD pathologies. The horizontal coordinate is the corresponding gene location within the chromosome (defined as the midpoint of the start and end positions). The vertical coordinate is the −log10 of the *p* value if the protein is associated with slower cognitive decline, that is, greater cognitive resilience and log10 of the *p* value if it is associated with faster cognitive decline, that is, less resilience. The dashed lines correspond to the reference significance level representing $\alpha = 5 \times 10^{-6}$. The red boxes denote proteins that are associated with more than 1 cognitive ability. The white boxes denote proteins that are specifically associated with the corresponding cognitive ability. ADRD = Alzheimer disease and related dementia.

5 were specific to single abilities. Figure 3 summarizes the frequencies of the shared and unique proteins across the 5 cognitive outcomes and global cognition.

Cognitive resilience proteins are associated with cognitive decline after controlling for ADRD pathologies. So, in further analyses, we estimated the percentage of the variance of cognitive decline accounted for by ADRD pathologies and by the cognitive resilience proteins identified in the current study. To estimate the variance of declining cognitive abilities accounted for by ADRD pathologies, we compared a model that included demographic covariates (age at death, sex, and education) with one with demographic covariates and 9 ADRD pathologies. The variance in cognitive decline

Figure 3 Frequency of Resilience Proteins Associated With Different Cognitive Abilities



This histogram shows the total number of identified resilience proteins (n = 47) from the dorsolateral prefrontal cortex after adjusting for age at death, sex, education, and 9 ADRD pathologies. The diagram illustrates the number of proteins, as represented by the bars, which were shared by specific cognitive abilities, as represented by the connected black dots. As illustrated in the figure, most cortical resilience proteins are general across different cognitive abilities, that is, providing resilience to more than 1 ability; however, there is evidence of specificity at the individual ability level, that is, specific proteins are differentially associated with different cognitive abilities, as indicated by the single (unconnected) dots. There were no proteins associated with resilience for visuospatial ability. ADRD = Alzheimer disease and related dementias.

accounted for by ADRD pathologies over and above demographic covariates ranged from 31% (episodic memory) to 14% (perceptual speed). When we added the cognitive resilience proteins to the models, these proteins explained up to 26% (for working memory) of additional variance in cognitive decline over and above demographic covariates and ADRD pathologies. For the rest of the cognitive abilities, these resilience proteins explained an additional 18% for episodic memory, 15% for global cognition and semantic memory, and 5% for perceptual speed of variance in cognitive decline over and above demographic covariates and ADRD pathologies (eTable 2, links.lww.com/WNL/D260).

NRN1 was a top protein associated with greater cognitive resilience (i.e., slower cognitive decline controlling for the effects of ADRD pathologies) in episodic memory, working memory, and slightly less so, with semantic memory (episodic memory: estimate = 0.029, SE = 0.004, $p = 5.8 \times 10^{-11}$; working memory: estimate = 0.021, SE = 0.004, $p = 1.2 \times 10^{-7}$; and semantic memory: estimate = 0.020, SE = 0.004, $p = 2.2 \times 10^{-6}$). When investigating global cognition, NRN1 was also associated with greater cognitive resilience (global cognition: estimate = 0.026, SE = 0.004, $p = 1.3 \times 10^{-11}$). The effects of NRN1 on episodic memory led up to an estimated 12% of slowing in decline after also accounting for pathology (estimate = 0.03, SE = 0.005, $p < 0.001$); the effects of this protein on other abilities ranged from 7% for semantic memory (estimate = 0.02, SE = 0.004, $p < 0.001$) to 11% for working memory (estimate = 0.02, SE = 0.004, $p < 0.001$). For global cognition, the decline was estimated to be 11% slower when NRN1 was added to the model (estimate = 0.03, SE = 0.003, $p < 0.001$), after controlling for the effects of pathology. We illustrate the effects afforded by NRN1 on the rate of cognitive decline for different cognitive abilities by comparing its effect on global cognitive function and on episodic, working, and semantic memory in Figure 4.

Biological Pathways That May Underlie Cognitive Resilience Proteins

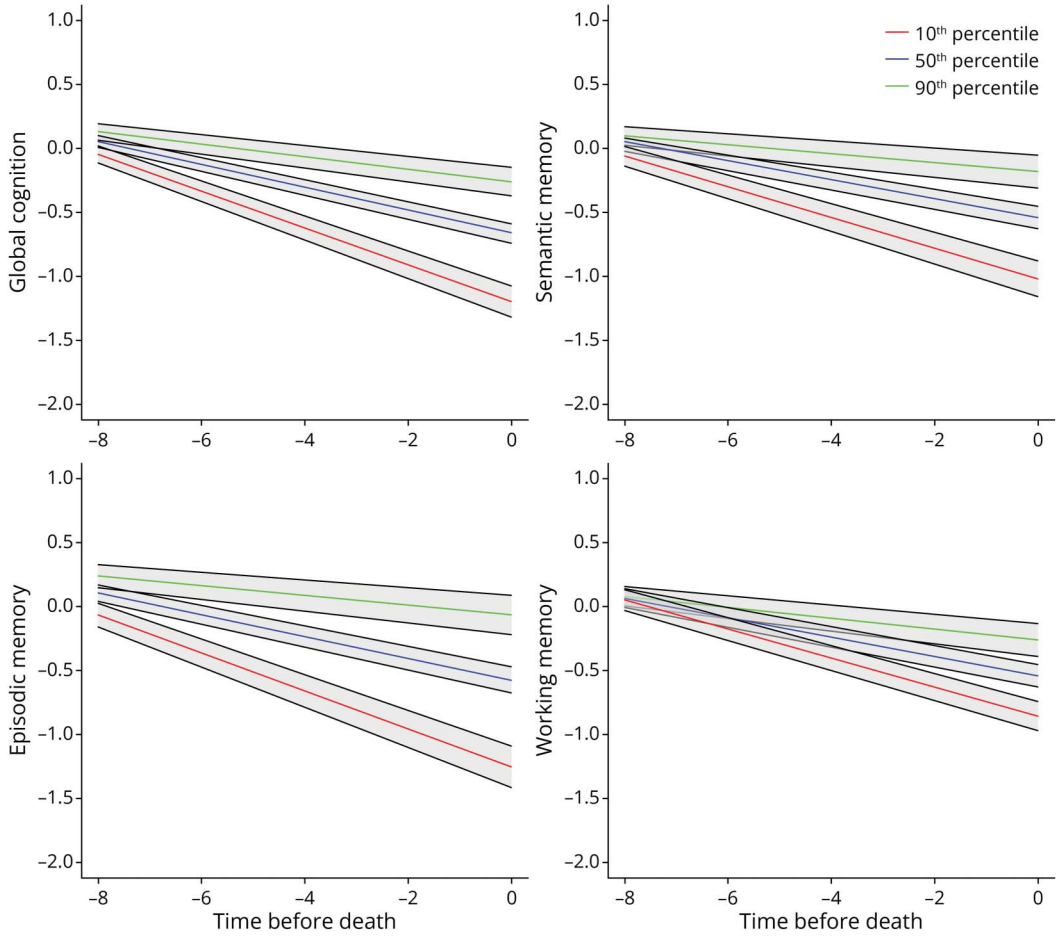
We conducted an exploratory GO analysis to identify candidate pathways that might underlie cognitive resilience. Proteins with $p < 0.05$ were considered as potentially associated with cognitive resilience (of 8,223 genes, we identified 843 genes associated with global cognition; 677 genes with episodic memory, 615 genes with working memory, 323 genes with semantic memory, 338 genes with perceptual speed, and 224 genes with visuospatial ability).

We identified 27 biological pathways associated with cognitive resilience in 1 or more cognitive abilities. We illustrate these pathways along with the number of overlapping proteins within each pathway for each cognitive ability in Figure 5. Results suggested that some pathways were differentially associated with some cognitive abilities.

Resilience proteins associated with global cognition and episodic memory shared 13 pathways, with the top 5 pathways for these abilities being enriched for translational termination and mitochondrial translation molecular mechanisms, suggesting that these mechanisms might contribute to cognitive resilience for these performances. By contrast, only 8 pathways were significantly (FDR of 0.05) associated with resilience proteins for working memory, and none of those pathways overlapped with pathways associated with global cognition or episodic memory. Pathways associated with working memory resilience proteins were driven by nuclear-transcribed messenger RNA (mRNA) catabolic process nonsense-mediated decay, regulation of vesicle-mediated transport, protein localization, and translational initiation.

To illustrate the differential associations of the biological processes enriched for the 2 main abilities that were associated with the most resilience proteins, we provide 2 network plots showing the top 5 GO terms and their resilience proteins for both episodic and working memory abilities in Figure 6. While a unique pathway was also identified for resilience proteins associated with semantic memory ability: the acetyl-CoA biosynthetic process from pyruvate, no other pathways were identified. No significant results were found for perceptual

Figure 4 Association of Expression Level of NRN1 With Different Cognitive Abilities and With Global Cognition



These panels represent model-derived trajectories of cognitive decline for an average participant (female, age 90 years and 15 years of education) illustrating the varied effects of level of NRN1 in the 10th, 50th, and 90th percentiles on slowing the rate of cognitive decline for different cognitive abilities including episodic memory, semantic memory, and working memory and a summary measure representing global cognition. As noted, the participant in the 10th percentile of NRN1 levels has a steeper decline than someone in the 90th percentile. Furthermore, the effects of low NRN1 might be more pronounced for episodic memory than any other ability, while high NRN1 might be more impactful for episodic memory than any other ability, which might stress that effects of NRN1 might be more pronounced *within* abilities than across them.

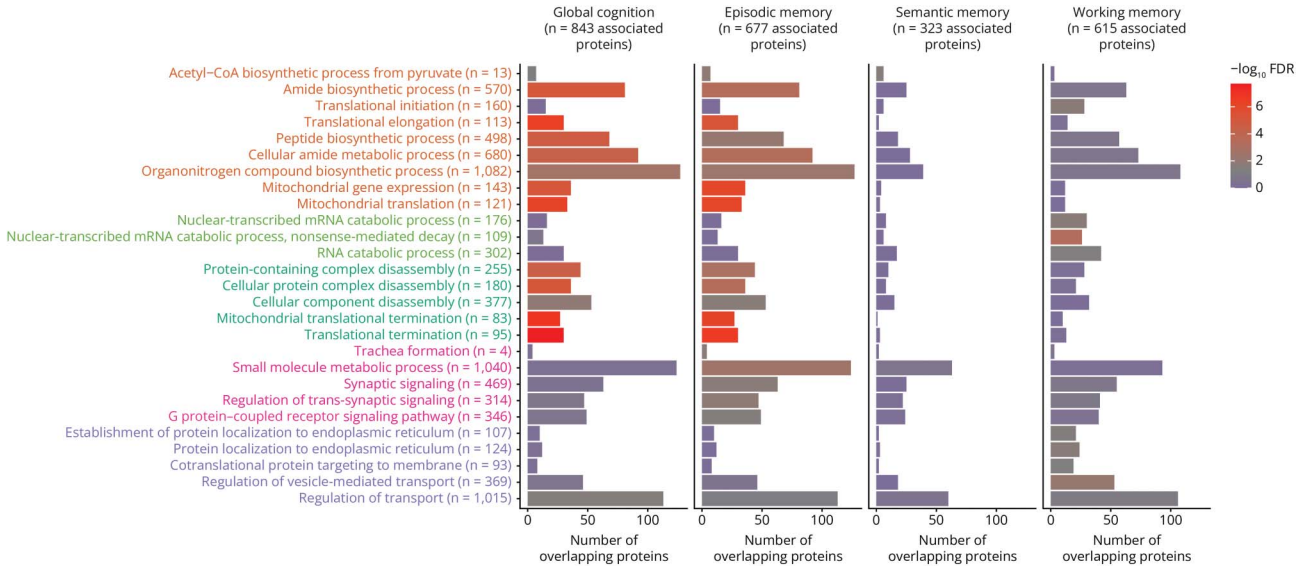
speed or visuospatial ability. Full details of the top 5 significant pathways (FDR ≤0.05) for global cognition, episodic memory, working memory, and semantic memory are provided in eTable 3 (links.lww.com/WNL/D260).

Discussion

Prior work using a composite measure for cognitive function identified cortical resilience proteins that account partly for the heterogeneity of late-life cognitive decline.[2] The current proteome-wide study of 604 deceased older adults extends these prior findings by showing that some cortical proteins are strongly associated with resilience of specific cognitive abilities and some cortical proteins are associated with resilience of multiple cognitive performances. Further analyses suggested that the varied associations of cortical resilience proteins may reflect both distinct and shared underlying biological pathways. These data provide the aging research community with a parsimonious set of high-value targets for further mechanistic and drug discovery studies that can catalyze the development of personalized medical treatments targeting specific cognitive deficits in aging adults.

Cognitive function is a complex phenotype consisting of varied but interrelated cognitive abilities that may differ in their rates of decline. A global cognitive measure is typically derived from multiple cognitive domains whereby each domain is derived from multiple cognitive tests. If we approach cognitive ability through a hierarchical factor structure, approximately a third of the variation of individual differences in cognitive decline has been reported to be due to change at the highest-order level, which is akin to a global composite level, another third is specific to cognitive change at the domain-specific level that reflects the various cognitive abilities, and yet, approximately another third is specific at the test level.[14]

Figure 5 Gene Ontology Analysis and Molecular Pathways That May Underlie Resilience for Different Cognitive Abilities



Each bar represents the number of overlapping proteins (listed on the x-axis) within each pathway (listed on the y-axis) for each cognitive ability as represented in the 4 vertical panels. The biological pathways on the y-axis are color coded according to their semantic clustering depending on their GO terms. The number of measured proteins in our dataset that were assigned to each pathway is given in parentheses after the pathway names (y-axis). Bars are color coded depending on the strength of the association, as depicted by the log-adjusted *p* value on the right of the chart. As can be seen in the chart, and depicted by red bars, the most significant associations between the resilience proteins and corresponding biological pathways were present in the global cognition and episodic memory abilities. The orange bars in the working memory panel illustrate that proteins identified within that ability were moderately associated with different pathways compared with those that were associated with episodic memory or global cognition, suggesting differential associations between cognitive abilities and underlying biological pathways. FDR = false discovery rate; GO = gene ontology; mRNA = messenger RNA.

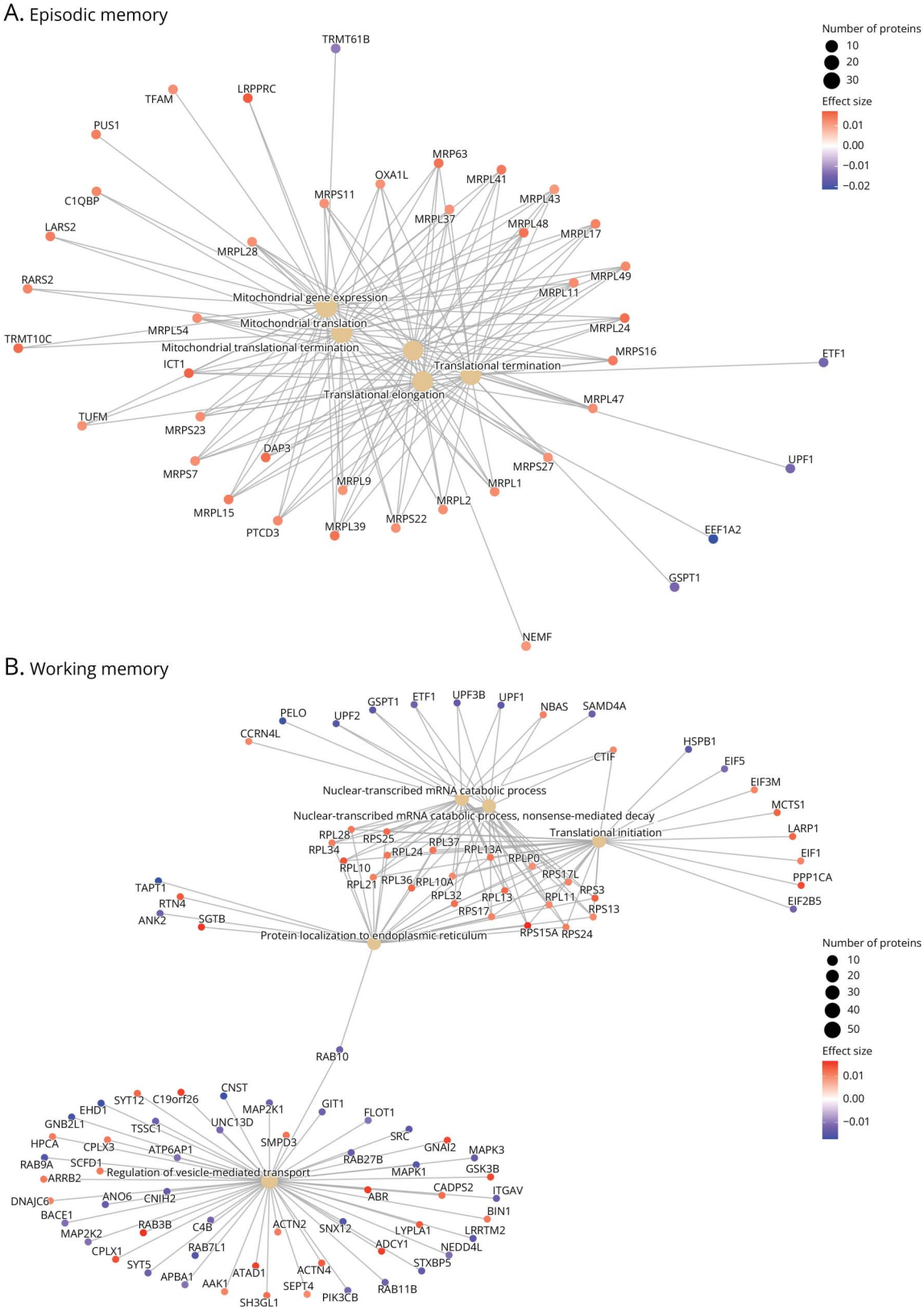
While composites, such as global cognition, are statistically more robust, they do not explain the total amount of variance in cognitive abilities; hence, at a more detailed level, some variance in individuals' performance is explained by very specific cognitive abilities.[32] Research has not yet addressed whether biological processes that might drive cognitive resilience at more specific levels of cognitive ability also provide resilience at a global level. To more fully leverage the proteome-wide analyses to inform on cognitive resilience proteins, we examined measures of global cognition and specific cognitive abilities.

The current study used data from a much larger sample that extends our prior study by identifying 47 cortical resilience proteins for global cognition and 4 different cognitive abilities.[2] While most proteins (n = 42) identified for specific cognitive abilities were shared with global cognitive ability, 5 proteins were associated with distinct abilities. As per strict Bonferroni threshold, our proteome-wide study identified 3 novel proteins associated with working memory (PSMB1, CLDN10, and TMEM245, also known as C9orf5) and 2 associated with semantic memory (CADPS2 and TMTM141). The identification of these proteins would have been overlooked if only a composite measure of cognitive function had been analyzed.

Our recent work has shown that multiple cortical proteins are associated with cognitive resilience and that everyone has some degree of resilience.[2,10] Results also suggested that some proteins are strong drivers of cognitive resilience and that multiple biological pathways are implicated in cognitive resilience.[2] The larger sample size and the study of different cognitive abilities in the current study identified additional proteins while simultaneously providing additional data that reinforce the notion that some proteins are strong drivers of cognition, that is, generalist genes may have diffuse effects within and between cognitive abilities due to their pleiotropic pathways. On the contrary, we also provide evidence that some proteins are differentially associated with specific cognitive abilities and that there may be a core number of proteins that drive distinct but related cognitive abilities. Cognitive aging literature supports both notions,[32,33] that is, both specificity and the generality of cognitive abilities. Thus, extending this work on cognitive resilience proteins may help in elucidating the molecular mechanisms underlying cognitive aging of diverse cognitive abilities. Consequently, efforts to target a "generalist" protein may boost cognitive abilities on a more widespread level, while targeting more "specific" proteins could improve a specific cognitive ability.

Of all the identified proteins, cortical neuritin (NRN1) was associated with cognitive resilience across multiple cognitive abilities, indicating its pleiotropic effects across related but different cognitive phenotypes. NRN1 was the strongest and only protein that was associated with resilience across 4 cognitive abilities. NRN1 is a neurotropic factor predominantly expressed in the brain, and in the hippocampus, and which promotes axon regeneration.[34] It plays an

Figure 6 Differential Associations of the Biological Pathways Enriched for Episodic and Working Memory Abilities



The network figures for episodic memory (panel A) and for working memory (panel B) show the top 5 pathways (yellow nodes) and the resilience proteins associated with these pathways. The color of the proteins reflects the effect size of the association with cognitive resilience. High abundance of a positively associated protein (red color) is associated with better cognitive abilities. As the figure shows, the top 5 pathways enriched for resilience proteins associated with episodic memory are different than the top 5 pathways enriched for resilience proteins associated with working memory, providing evidence that different processes might be driving these related but separate abilities.

important role in synaptic function and plasticity and in regulating neural function.[35] Neuritin has been previously prioritized as a hub that coexpressed with a community of proteins with high correlation to cognitive stability and is known for important roles in synaptic maturation and neuroprotective mechanisms.[36-39] In 2 previous studies, NRN1 was also associated with a slower rate of cognitive decline after adjustment for the effects of ADRD pathologies.[2,3] Although in our previous study[2] the sample size was smaller than the current study, the consistency of the NRN1 as a top protein remained.

In AD dementia studies, results have also shown that the temporal unfolding of cognitive decline across domains starts with episodic memory, progressing to frontal executive function, and at later stages, to semantic memory.[17] Our results showed that distinct GO pathways are implicated in episodic memory, working memory, and semantic memory. While for episodic memory the pathways involved were related to the prevention of inappropriate transcription of downstream mitochondrial genes, pathways for working memory related more to protein regulation and localization, while the 1 pathway identified for semantic memory was again related to energy in the mitochondria. It might be possible that while these cognitive abilities are correlated, they also draw on different pathways during later life as aging and disease processes unfold. Clinical-pathologic studies have supported this evidence, in that temporally, episodic memory decline has been shown to be the first cognitive ability in the presence of various neurodegenerative pathologies accumulating in aging brains, with other cognitive phenotypes showing distinct differential associations with varied ADRD pathologies.[18]

Unique GO pathways that are involved in the prevention of the translation of mRNA into potentially harmful proteins, and pathways involved in the modulation of the rate, frequency, and extent of the directed movement of substances into and within cells, were identified for the working memory domain. Similarly, our semantic memory domain that was composed of tests that involved generating words in response to pictorial and semantic stimuli and a test that required reading aloud a series of 20 atypical sounding words is believed to derive from cognitive networks located in the medial temporal lobes, including the hippocampus and the dentate gyrus. Indeed, the expression of CADPS2, which was only associated with semantic memory in our study, has previously been found to be restricted to certain brain regions, including the CA1/CA2 regions of the hippocampus, dente gyrus, the olfactory bulb, and the cerebellum.[40] The abundance of CAPS proteins in the synaptic terminal has indicated that they may be important for neuronal function.[41] Furthermore, a unique pathway relating to metabolism in the mitochondria for semantic memory was also identified.

Our study is limited in that our participants do not represent a diverse population, in that the majority are non-Latino White individuals with an above-average education. Thus, these findings will need to be replicated in diverse cohorts. We controlled for 9 common neuropathologic indices, but other pathologies that were not measured (e.g., white matter abnormalities) may account for additional unexplained variance in cognitive decline. This proteome-wide discovery study of a single brain region (the DLPFC) identified resilience proteins associated with different cognitive abilities. Further research will need to validate these findings and determine the regional specificity of the resilience proteins identified in this study. While our studies[10,42] suggest that most proteins are pleiotropic, by obtaining proteome from other regions in the brain, the distribution of associations between cortical proteins and cognitive abilities might look different than our current results; conversely, more pathways might be identified. Future work is needed to map the biological landscape that underlies the various abilities across multiple brain regions, which may inform on the molecular mechanisms driving different locations within distributed cognitive networks. The identified proteins in this study offer a glimpse into potential pathways that are underlying resilience across cognitive abilities; proteins that did not make the adjusted Bonferroni threshold might still play important yet unidentified roles across the different abilities. Further analytic approaches that reduce a large number of proteins may be useful to identify important proteins that were excluded because of the strict correction applied for multiple testing.

Strengths of the study include our focus on the proteomics of cognitive resilience in older adults from 2 well-characterized population-based cohort studies. Participants were free of dementia at baseline and had measured trajectories of cognitive decline over multiple years across varied cognitive abilities. Our proteome-wide study associated thousands of high-abundance cortical proteins to cognitive resilience across multiple cognitive performances over many years before death while controlling for diverse ADRD pathologies. This is a comprehensive approach for identifying protein signals associated with cognitive resilience. We used Bonferroni correction for multiple testing, which reduces the chance of spurious findings. All participants in this study took the same tests with the same instruction as in our previous studies. By increasing the statistical power of the study and by exploring different cognitive abilities, we were able to discover additional cognitive resilience proteins.

Our study suggests that different proteins and underlying biological pathways were associated with cognitive resilience across both specific and shared cognitive abilities. Our previous work has shown that the combination of neuropathologies is highly heterogeneous across individuals,[43-45] and therapies are not currently available for most neuropathologies. Thus, our findings may have important translational consequences and inform on further drug discovery to develop therapies targeting resilience proteins that can maintain cognitive function and brain health in older adults even in the presence of currently untreatable ADRD pathologies.

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Disclosure

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