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RESEARCH ARTICLE

Neuropathologic Correlates of Human Cortical Proteins in Alzheimer Disease and Related Dementias

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Abstract

Background and Objectives

Alzheimer dementia is a complex clinical syndrome that can be defined broadly as an amnestic multidomain dementia. We previously reported human cortical proteins that are implicated in Alzheimer dementia. To understand the pathologic correlates of these proteins for underlying disease mechanisms, we investigated cortical protein associations with common age-related neuropathologies.

Methods

Participants were community-dwelling older adults from 2 cohort studies of aging and dementia. All underwent detailed annual clinical evaluations, and brain autopsies were performed after death. We use Alzheimer disease (AD) to refer to pathologically defined disease and Alzheimer dementia to refer to the clinical syndrome. Indices for AD, cortical Lewy bodies, limbic predominant age-related TAR DNA binding protein *43* encephalopathy neuropathologic changes (LATE-NC), hippocampal sclerosis, macroscopic infarcts, microinfarcts, cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis were quantified during uniform structured neuropathologic evaluations. High-throughput protein abundances from frozen dorsolateral prefrontal cortex were quantified with mass spectrometry–based tandem mass tag proteomics analysis. Eleven human cortical proteins implicated in Alzheimer dementia, including angiotensin-converting enzyme, calcium-regulated heat-stable protein 1 (CHSP1), procathepsin H (CATH), double C2-like domain-containing protein α, islet cell autoantigen 1–like protein, serine β-lactamase–like protein LACTB, mitochondrial, pleckstrin homology domain–containing family A member 1, replication termination factor 2, sorting nexin-32, syntaxin-4, and syntaxin-6 (STX6), were previously identified with an integrative approach. Logistic regression analysis examined the association of protein expression with each of the neuropathologic indices.

Results

A total of 391 older adults were included. We did not observe associations of these protein targets with pathologic diagnosis of AD. In contrast, multiple proteins were associated with non-AD neurodegenerative and cerebrovascular conditions. In particular, higher CHSP1 expression was associated with cortical Lewy bodies and macroscopic infarcts, and higher CATH expression was associated with LATE-NC and arteriolosclerosis. Furthermore, while higher STX6 expression increased the risk of Alzheimer dementia, the protein was not associated with any of the neuropathologic indices investigated.

Discussion

Cortical proteins implicated in Alzheimer dementia do not necessarily work through AD pathogenesis; rather, non-AD neurodegenerative and vascular diseases and other pathways are at play. Furthermore, some proteins are pleiotrophic and associated with both neurodegenerative and cerebrovascular pathologies.

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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

ACE = angiotensin-converting enzyme; **AD** = Alzheimer disease; **CATH** = procathepsin H; **CHSP1** = calcium-regulated heat-stable protein 1; **CI** = confidence interval; **DOC2A** = double C2-like domain-containing protein α; **GIS** = global internal standard; **GWAS** = genome-wide association study; **H&E** = hematoxylin & eosin; **ICA1L** = islet cell autoantigen 1–like protein; **LACTB** = serine β-lactamase–like protein LACTB, mitochondrial; **LATE-NC** = limbic-predominant age-related TDP-43 encephalopathy neuropathologic changes; **NIA** = National Institute on Aging; **OR** = odds ratio; **ROSMAP** = Religious Orders Study and Rush Memory and Aging Project; **SNX32** = sorting nexin-32; **STX4** = syntaxin-4; **STX6** = syntaxin-6; **TDP-43** = TAR DNA binding protein 43.

Alzheimer dementia is a complex clinical syndrome that can be defined broadly as an amnestic multidomain dementia.[1] The number of older Americans with Alzheimer dementia is expected to increase dramatically to reach ≈14 million by 2050.[2] The financial and health impacts of Alzheimer dementia on families and society are enormous.[3] Discovery of therapeutic targets for Alzheimer dementia has never been more urgent. Proteins play a key role in various molecular functions and biological processes and are among the most common targets of pharmacologic manipulation for complex human diseases. Historically, Alzheimer disease (AD) trials for disease-modifying agents, including the most recent Food and Drug Administration–approved drug aducanumab, have been directed predominantly against β-amyloid protein and to a lesser extent tau protein.[4] However, the success rates of these trials are disappointingly low. Studies suggest that novel protein targets with direct genetic support are more likely to succeed in drug development, particularly in phase 2 or 3.[5,6] Building on this idea, we previously reported 1,139 proteins with evidence of heritable expression in the human dorsolateral prefrontal cortex, of which 11 proteins were identified as being implicated in Alzheimer dementia.[7] These novel protein signals provide important opportunities for future mechanistic research.

Of note, Alzheimer dementia is attributable to a host of neuropathologic conditions that commonly coexist in the aging brain.[8,9] We and others have reported that, while AD pathology is the main driver of Alzheimer dementia, other non-AD neurodegenerative and cerebrovascular diseases also play an important role.[10,11] Therefore, without a solid understanding of underlying neuropathologic correlates, protein targets implicated in Alzheimer dementia lack specificity.

To fill this knowledge gap, we leveraged proteomic and clinicopathologic data from nearly 400 older adults and investigated the associations of these 11 cortical proteins with common neuropathologies. We first investigated the association of these cortical proteins with clinical diagnosis proximate to death. Next, for each protein separately, we examined the associations with 9 common neuropathologic indices, including those of AD and non-AD neurodegenerative and cerebrovascular conditions. Finally, we explored the extent to which protein association with Alzheimer dementia is explained by these neuropathologic indices. Power calculations were performed to inform the sample size needed for a larger study.

Methods

Study Participants

Participants came from 2 ongoing cohort studies of aging and dementia, the Religious Orders Study and Rush Memory and Aging Project (ROSMAP).[12] ROSMAP recruits community-dwelling older adults free of known dementia. All participants were followed up longitudinally until death. Detailed clinical evaluations were administered each year at the participant's home, and brain autopsy was performed after death.

Alzheimer Dementia Diagnosis

Annual detailed clinical evaluations include an interview on medical history, a neurologic examination, a comprehensive cognitive performance testing, and a medication inspection. Results for cognitive testing were scored by computer algorithm and reviewed by a neuropsychologist for the presence of cognitive impairment. Diagnosis of Alzheimer dementia was made by a trained clinician each year according to the criteria of the joint working group of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.[13] The diagnosis requires a history of cognitive decline and impairment in multiple cognitive domains that include memory. After a participant died, all available clinical information was reviewed by a board-certified neurologist, and a final diagnosis regarding the participant's cognitive status was provided, blinded to findings from the neuropathologic evaluation.[14]

Neuropathologic Evaluations

Brain autopsy was performed on average 8 hours (SD 5.5 hours) postmortem. Brain was removed, weighed, and cut coronally into 1-cm slabs. One hemisphere with more visible pathologies was fixed in 4% paraformaldehyde, and the other hemisphere was rapidly frozen at −80°C. Neuropathologic evaluations were conducted on fixed tissue by investigators blinded to all clinical information. Nine common age-related neuropathologic conditions, including AD, Lewy bodies, limbic-predominant age-related TAR DNA binding protein 43 (TDP-43) encephalopathy neuropathologic changes (LATE-NC), hippocampal sclerosis, macroscopic infarcts, microinfarcts, cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis, were assessed following a uniform structured procedure.

Pathologic AD diagnosis was determined following the recommendation of the National Institute on Aging (NIA)–Reagan criteria.[15] All cases received a pathologic diagnosis of no AD, low-likelihood AD, intermediate-likelihood AD, or high-likelihood AD based on the Braak score for neurofibrillary tangles and the *Consortium to Establish a Registry for Alzheimer's Disease* estimate of neuritic plaques. Individuals with intermediate or high likelihood were classified as having a pathologic AD diagnosis. While we recently implemented the new ATN criteria,[16] we have >1,800 brains collected starting in 1994, and not all have been classified with the ATN criteria as of yet. In contrast, the NIA-Reagan criteria are currently available for all autopsies. We compared the 2 diagnoses using data from cases that had both NIA-Reagan and ATN. Notably, they are highly concordant, with a Cohen κ as high as 0.93. This suggests that our findings are unlikely to differ regardless of which diagnostic criteria are used.

The presence of cortical Lewy bodies in midfrontal, temporal, or inferior parietal cortices was determined with antibodies to phosphorylated α-synuclein.[17] TDP-43 pathology was assessed in amygdala, hippocampus, dentate gyrus, entorhinal cortex, and midfrontal and middle temporal cortices with the use of antibodies to phosphorylated TDP-43 and summarized into stage 0 (no presence of TDP-43), stage 1 (TDP-43 localized to the amygdala), stage 2 (extension of TDP-43 to the hippocampus or entorhinal cortex), and stage 3 (extension into the neocortex). Of note, the staging is slightly different from the LATE-NC working group recommendation[18] such that TDP43 inclusion in midfrontal gyrus is not required for stage 3. In this study, we dichotomized the measure by collapsing stage 0 with 1 and stage 2 with 3. Hippocampal sclerosis refers to severe neuronal loss and gliosis in CA1 or the subiculum, and the presence of hippocampal sclerosis was determined by examining hematoxylin & eosin (H&E)–stained sections of midhippocampus.[19]

Five cerebrovascular conditions were assessed. Macroscopic infarcts were identified during gross examination and confirmed histologically, and microinfarcts were examined with the use of H&E–stained sections of at least 9 brain regions.[20] Cerebral amyloid angiopathy was assessed in midfrontal, midtemporal, angular, and calcarine cortices with antibodies to β-amyloid.[21] For each brain region, amyloid deposition in meningeal and parenchymal vessels was scored. Region-specific scores were averaged and summarized into a semiquantitative rating of none, mild, moderate, and severe. Vertebral, basilar, posterior cerebral, middle cerebral, and anterior cerebral arteries and proximal branches in the circle of Willis were inspected for atherosclerosis during gross examination. H&E–stained sections of anterior basal ganglia was assessed for arteriolosclerosis. For both vessel diseases, severity of vessel wall thickening was graded into a semiquantitative rating of none, mild, moderate, and severe.[20] In this study, we focused on moderate or severe amyloid angiopathy, atherosclerosis, and arteriolosclerosis.

Mass Spectrometry–Based Tandem Mass Tag Proteomics Analysis

We performed high-throughput proteomic sequencing by conducting isobaric tandem mass tag peptide labeling, liquid chromatography, and mass spectrometry using brain tissues from dorsolateral prefrontal cortex of 400 deceased ROSMAP participants.[22,23] Briefly, brain tissues were homogenized for protein digestion. Lysates samples, together with 100 global internal standard (GIS) mixtures, were randomly assigned into 50 batches for tandem mass tag labeling. After labeling, high-pH fractionation was performed. Fractions obtained were loaded onto the liquid chromatography–tandem mass spectrometry platform for mass spectrometry analyses. Raw files were analyzed with the Proteome Discoverer suite (version 2.3). Spectral assignment was performed by searching against the UniProtKB human proteome database (February 2019). Peptides were assembled into proteins, and reporter ions were quantified.

Quantified protein expression underwent stringent quality controls at both the protein and sample levels. Within each batch, protein expression was checked against the GIS and set to missing if it fell outside the 95% confidence interval (CI) of the GIS. Proteins with excessive missing values (>50%) were removed. Separately, sample outliers were identified and removed. Individual protein expression was scaled and log2 transformed. Technical confounders such as sequencing batch were regressed out. After the quality controls, data for 8,356 proteins in 391 samples were retained.

Identification of Cortical Proteins in Alzheimer Dementia

Details on identifying cortical proteins in Alzheimer dementia were described elsewhere.[7] Briefly, we identified proteins with an abundance that was *cis*-regulated by genetic variants implicated in Alzheimer dementia according to a recent genome-wide association study (GWAS) meta-analysis.[24] First, we identified 1,475 heritable cortical proteins using the FUSION pipeline.[25] Then we estimated the effects of neighboring single nucleotide polymorphisms of a gene on its protein abundance and integrated those results with summary statistics from the GWAS meta-analysis. This approach may be thought of as performing a gene-based test of association with Alzheimer dementia, weighting each single nucleotide polymorphism by its association with *cis*-regulated protein abundance. Next, we applied COLOC[26] and summary data–based mendelian randomization[27] to test for proteins with evidence of a causal role in Alzheimer dementia. Eleven cortical proteins were identified: angiotensin-converting enzyme (ACE), calcium-regulated heat-stable protein 1 (CHSP1), procathepsin H (CATH), double C2-like domain-containing protein α (DOC2A), islet cell autoantigen 1–like protein (IAC1L), serine β-lactamase–like protein LACTB, mitochondrial (LACTB), pleckstrin homology domain–containing family A member 1, replication termination factor 2, sorting nexin-32 (SNX32), syntaxin-4 (STX4), and syntaxin-6 (STX6). For the current analyses, we targeted these 11 proteins to examine their associations with neuropathologies.

Statistical Analysis and Power Calculation

Demographic, clinical, and neuropathologic characteristics of the study participants were described, and differences by Alzheimer dementia status were compared by use of the Student *t*, χ^2, and Wilcoxon rank-sum tests as appropriate. The protein associations with Alzheimer dementia and neuropathologies were examined with logistic regression models. In these models, Alzheimer dementia diagnosis and individual neuropathologic indices were binary outcomes, and each of the 11 proteins were analyzed separately as a predictor. All the models were controlled for age, sex, and education. Unless otherwise noted, statistical significance was determined at a nominal α level of 0.05.

To assess potential futility of associations, we conducted power calculation to estimate the sample size required to detect observed effect sizes (i.e., odds ratios [ORs] with every 1-SD increase in the expression level of a protein) with 80% power. The prevalence rate of individual outcome for the power calculation was based on the sample characteristics. We note that there was no appreciable difference in these rates between the sample and the overall ROSMAP cohorts.

Statistical analyses were performed with SAS/STAT software, version 15.2 (Cary, NC) and R programs, version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Power calculation was performed with PASS software, version 08.0.13 (Kaysville, UT).

Standard Protocol Approvals, Registrations, and Patient Consents

The studies were approved by an institutional review board at Rush University Medical Center. All participants provided informed consent for participation, an Anatomical Gift Act for organ donation, and a repository consent for data and biospecimen sharing for research purposes.

Data Availability

Data used in this study are available on request via Rush Alzheimer's Disease Center Resource Sharing Hub.

Results

Characteristics of Study Participants and Neuropathologic Burden

Of the 391 deceased older adults included in this study, 273 (69.8%) were female. The mean years of education was 15.8 (SD 3.6 years, range 5–28 years). On average, participants died at the age of 89 years (SD 6.5 years, range 65.9–106.5 years). The median Mini-Mental State Examination score proximate to death was 26 (Interquartile range 20–28), and approximately a third (31.9%) were diagnosed with Alzheimer dementia. Individuals who died with Alzheimer dementia tended to be older (Table 1).

The neuropathologic burden represented in these participants was similar to that observed in the overall ROSMAP cohorts. More than 90% of the sample, that is, 358 of 391, showed ≥1 neuropathologic conditions at autopsy. Approximately 60% met NIA-Reagan criteria for pathologic AD. Cortical Lewy bodies were present in 14.4%; 26.6% had LATE-NC; and 8.2% had hippocampal sclerosis. Cerebrovascular conditions were common, with chronic macroscopic and microinfarcts present in 32.0% and 28.1% of the sample, respectively. Moderate to severe amyloid angiopathy, atherosclerosis, and arteriolosclerosis were present in 26.7%, 31.0%, and 30.4%, respectively. Consistent with previous reports,[14,28] these neuropathologies commonly coexisted (Figure 1). Only 20% of the individuals had a single neuropathologic condition, while approximately half (49.4%) had ≥3. Among individuals with Alzheimer dementia, neurodegenerative conditions, including AD, LATE-NC, hippocampal sclerosis, and Lewy bodies, were much more common compared to cerebrovascular diseases (Table 1). Furthermore, individuals with Alzheimer dementia also showed more mixed pathologies. Almost all (>93%) of the Alzheimer dementia cases had multiple neuropathologies compared to 61% among those without dementia ($\chi^2_1 = 43.7$, $p < 0.001$).

Table 1 Characteristic of Study Participants

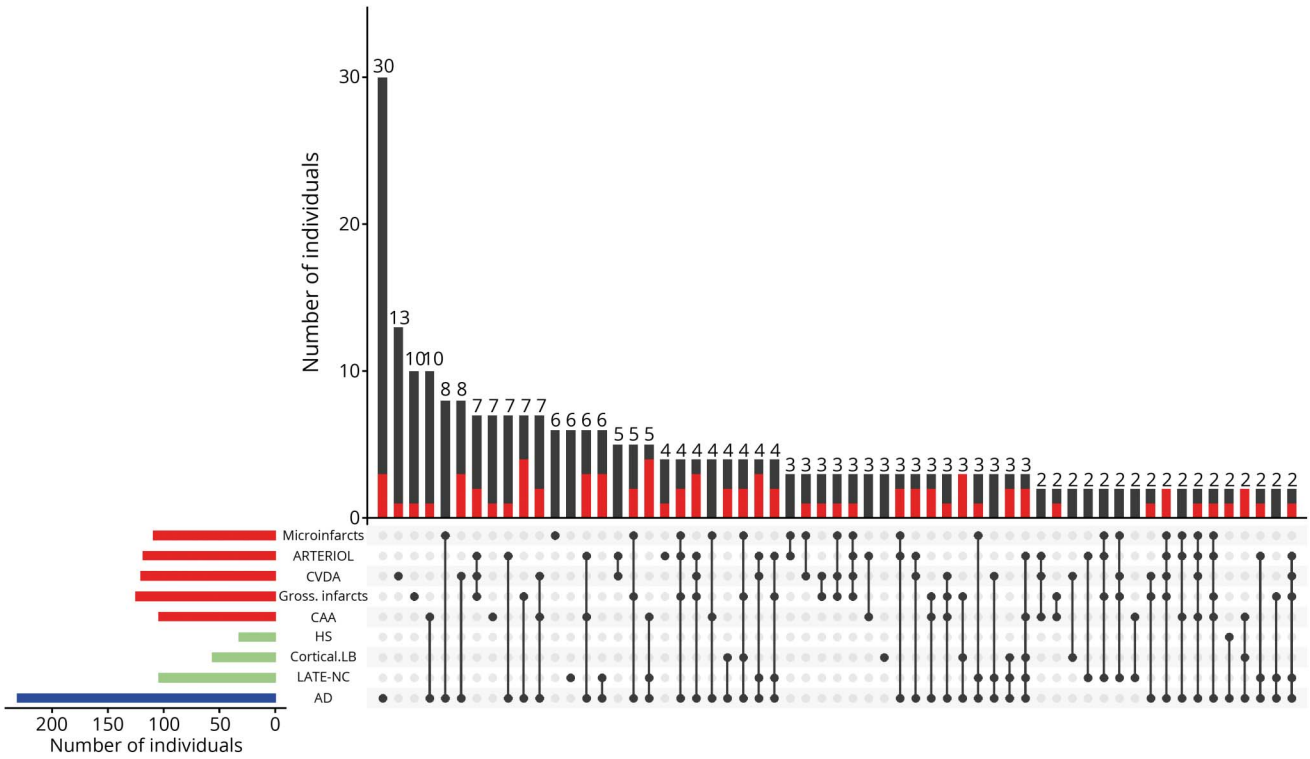
	No dementia	Alzheimer dementia	*p* Value
Age at death, y	88.0 (6.4)	92.2 (5.7)	<0.001[a]
Male sex, n (%)	85 (32.6)	29 (23.8)	0.079[b]
Education, y	15.7 (3.5)	15.9 (3.6)	0.711[a]
MMSE score	28 (26–29)	15 (6–20)	<0.001[c]
Pathologic AD diagnosis, n (%)	128 (49.0)	99 (81.2)	<0.001[b]
LATE-NC, n (%)	47 (18.0)	56 (45.9)	<0.001[b]
Hippocampal sclerosis, n (%)	9 (3.5)	22 (18.0)	<0.001[b]
Cortical Lewy bodies, n (%)	19 (7.3)	35 (28.7)	<0.001[b]
Macroscopic infarcts, n (%)	64 (24.5)	57 (46.7)	<0.001[b]
Microinfarcts, n (%)	69 (26.4)	39 (32.0)	0.262[b]
Cerebral amyloid angiopathy, n (%)	62 (23.9)	41 (33.6)	0.047[b]
Atherosclerosis, n (%)	72 (27.6)	45 (36.9)	0.066[b]
Arteriolosclerosis, n (%)	65 (25.2)	49 (40.2)	0.003[b]
No. of mixed pathologies	2 (1–3)	4 (3–5)	<0.001[c]

Abbreviations: AD = Alzheimer disease; MMSE = Mini-Mental State Examination; LATE-NC = limbic-predominant age-related TAR DNA binding protein *43* encephalopathy neuropathologic changes.
Statistics are mean (SD), number (percent), or median (interquartile range).
[a] *t* Test.
[b] Chi-squared test.
[c] Wilcoxon rank-sum test.

Association of Cortical Proteins With Alzheimer Dementia

First, we examined the associations of each of the 11 cortical proteins with Alzheimer dementia diagnosis proximate to

Figure 1 Mixed Pathologies in the Study Participants



Burden of mixed pathologies of the study participants is illustrated. Bar chart in the lower left corner shows the frequencies of individual neuropathologic indices. Connected black dots on the x-axis indicate the specific combination of neuropathologies represented (top 55 combinations shown). Histograms in the main panel show the frequencies of the neuropathologic indices for persons with and without Alzheimer dementia (red = present vs black = absent), ordered by overall frequency. AD = Alzheimer disease; Arteriol = arteriolosclerosis; CAA = cerebral amyloid angiopathy; CVDA = atherosclerosis; HS = hippocampal sclerosis; LB = Lewy bodies; LATE-NC = limbic-predominant age-related TAR DNA binding protein *43* encephalopathy neuropathologic changes.

death. In logistic regression models controlling for age, sex, and education, 5 of the 11 proteins showed nominal association with Alzheimer dementia (Table 2). Two protein associations survived the correction for multiple testing. A 1-SD increase in the CHSP1 expression level was associated with a 70% increase in the odds of having Alzheimer dementia (OR 1.73, 95% CI 1.35–2.22). Separately, a 1-SD increase in the STX6 expression level was associated with a 40% increase in the odds of having Alzheimer dementia (OR 1.40, 95% CI 1.11–1.77).

To further examine whether the observed associations of the 2 proteins differed by demographics, we augmented the models by adding terms for the 2-way interactions between the protein and demographics. It is interesting to note that while higher CHSP1 expression was associated with greater odds of having Alzheimer dementia in both sexes, the association was stronger in men (eTable 1, links.lww.com/WNL/B724). We did not find evidence that the STX6 association differs by age, sex, or education (eTable 2).

Association of Cortical Proteins With Neurodegenerative Conditions

Because these cortical proteins are nominated as targets for AD pathogenesis,[7] we examined the protein associations with pathologic AD diagnosis. None of the 11 proteins were associated (Figure 2). Next, we examined the protein associations with non-AD neurodegenerative indices, including cortical Lewy bodies, LATE-NC, and hippocampal sclerosis. In relation to cortical Lewy bodies, 2 proteins, SNX32 and CHSP1, showed nominal associations, of which CHSP1 survived the correction for multiple testing (OR 1.64, 95% CI 1.19–2.25). We did not observe significant interactions between CHSP1 and demographics in relation to cortical Lewy bodies (eTable 3, links.lww.com/WNL/B724). Separately, 4 proteins, STX4, CATH, DOC2A, and ICA1L, showed nominal association with LATE-NC, of which the CATH protein survived the multiple testing correction (OR 1.54, 95% CI 1.18–2.02). There was no significant interaction between CATH and demographics in relation to LATE-NC (eTable 4). None of the protein associations with hippocampal sclerosis reached nominal significance.

Association of Cortical Proteins With Cerebrovascular Conditions

The protein associations with the 5 cerebrovascular indices (i.e., chronic macroscopic infarcts, microinfarcts, amyloid angiopathy, atherosclerosis, and arteriolosclerosis) were relatively weaker compared to those with non-AD degenerative conditions. While none survived the correction for multiple

Table 2 Cortical Proteins With Alzheimer Dementia

UniProtKB	Protein	Estimate	SE	*p* Value
P12821	ACE_HUMAN	0.283	0.117	0.015
Q9Y2V2	CHSP1_HUMAN	0.548	0.127	<0.001
P09668	CATH_HUMAN	0.170	0.123	0.166
Q14183	DOC2A_HUMAN	0.100	0.116	0.389
Q8NDH6	ICA1L_HUMAN	−0.269	0.117	0.022
P83111	LACTB_HUMAN	−0.096	0.112	0.392
Q9HB21	PKHA1_HUMAN	0.206	0.120	0.085
Q9BY42	RTF2_HUMAN	0.158	0.123	0.199
Q86XE0	SNX32_HUMAN	−0.146	0.118	0.217
Q12846	STX4_HUMAN	0.252	0.121	0.038
O43752	STX6_HUMAN	0.339	0.120	0.005

Abbreviation: SE = standard error.
Estimates are log odds ratios, and results are from separate logistic regression models controlled for demographics.

testing, several nominally significant signals were identified. Briefly, the protein levels of ACE and CHSP1 were associated with greater odds of having macroscopic infarcts (OR 1.32, 95% CI 1.07–1.65 and OR 1.29, 95% CI 1.04–1.62 respectively). STX4 was associated with greater odds of having moderate to severe amyloid angiopathy (OR 1.30, 95% CI 1.03–1.64), while DOC2A showed an inverse association (OR 0.78, 95% CI 0.61–0.98). Finally, CATH was nominally associated with moderate to severe arteriolosclerosis (OR 1.36, 95% CI 1.07–1.72).

Notably, our data suggest that the CATH protein was associated with LATE-NC and, to a lesser extent, arteriolosclerosis. Emerging literature has shown that both pathologies are implicated in hippocampal sclerosis of aging.[19,29,30] Therefore, we examined the coexistent pattern of LATN-NC, hippocampal sclerosis, and arteriolosclerosis (eFigure 1, links.lww.com/WNL/B724). As expected, LATE-NC and hippocampal sclerosis were highly correlated with each other such that older adults with TDP43 pathology extending beyond the amygdala were more likely to have hippocampal sclerosis (χ^2_1 = 53.3, p < 0.001). We also observed a similar but weaker correlation between LATE-NC and arteriolosclerosis such that older adults with TDP43 pathology extending beyond the amygdala were more likely to have moderate or severe arteriolosclerosis (χ^2_1 = 5.45, p < 0.020). Together, these data lend further support that these pathologies are closely interrelated in aging brain.

Protein Association With Alzheimer Dementia Controlling for Neuropathologies

Of the 5 cortical proteins nominally associated with clinical diagnosis, we did not find a significant association of STX6 with any of the 9 neuropathologic indices investigated here. In addition, ACE was associated with macroscopic infarcts, CHSP1 was associated with both cortical Lewy bodies and macroscopic infarcts, ICA1L was associated with LATE-NC, and STX4 was associated with LATE-NC and amyloid angiopathy. We explored the extent to which these protein associations with Alzheimer dementia may work through common neuropathologies. We repeated the logistic regression analyses for the associations of these proteins with Alzheimer dementia by adding terms for neuropathologic indices associated with corresponding proteins. After controlling for these neuropathologies, the protein associations were indeed attenuated to varying degrees (Table 3). Specifically, the associations of ACE and CHSP1 with Alzheimer dementia persisted, while the results for ICA1L and STX4 were no longer significant.

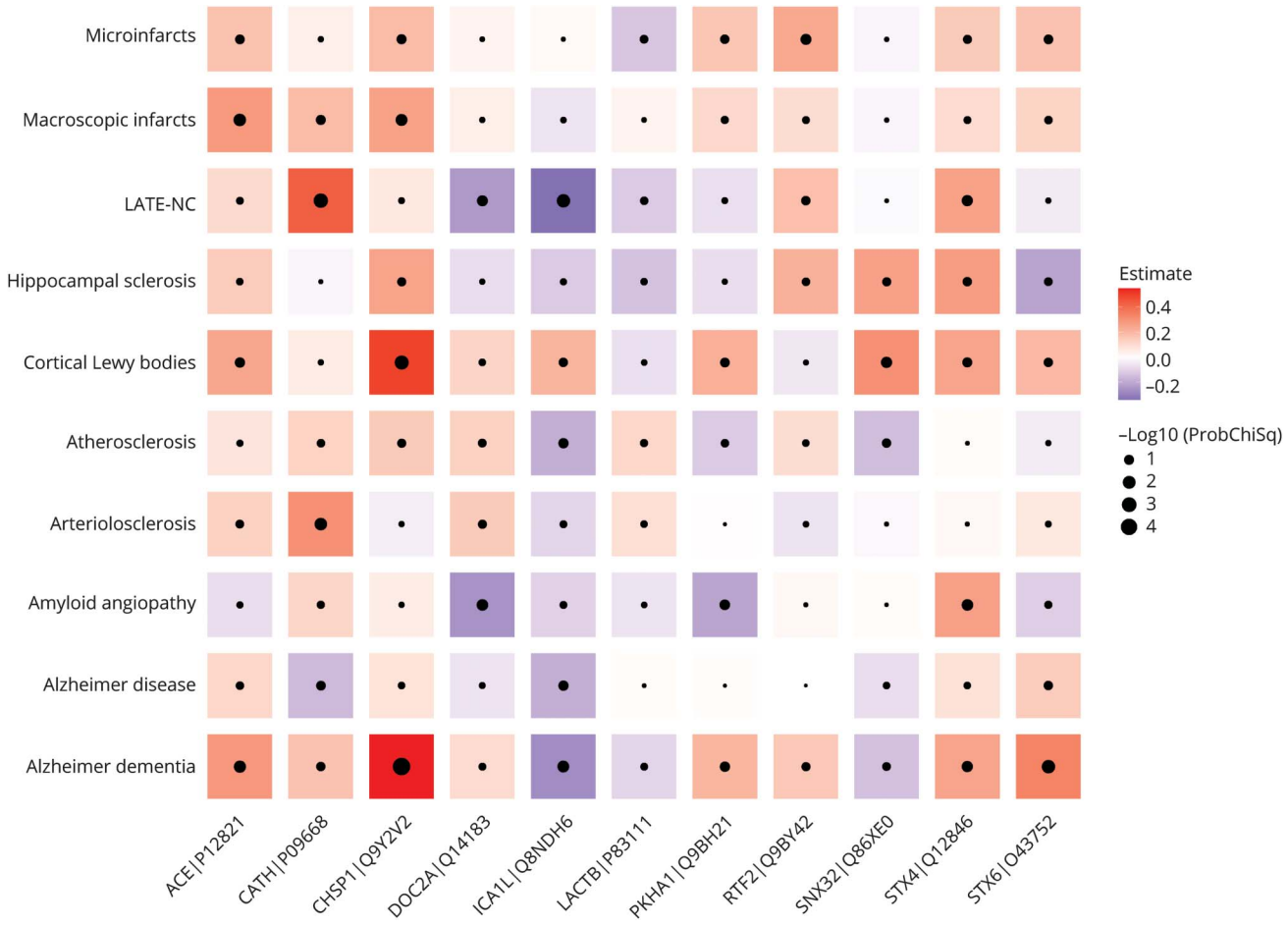
Power for Protein Associations With Alzheimer Dementia and Neuropathologies

To inform on the statistical power for larger analyses against futility, we performed power calculations to estimate the sample size required for detecting a protein association with Alzheimer dementia. The observed prevalence rate of Alzheimer dementia was 32% proximate to death, and the effect sizes (i.e., OR) for individual proteins in the sample ranged between 1.10 and 1.73, with an average of 1.27. At an α level of 0.05, the sample size required to detect an OR of 1.27 with 80% power was ≈630, but a sample of nearly 4,000 was needed to detect an OR as small as 1.10. Overall, a majority of these protein associations with Alzheimer dementia would be confirmed with a reasonably large sample size.

Next, we estimated the sample sizes required to detect protein association with AD and non-AD neurodegenerative conditions. In relation to AD, the observed effect sizes for a majority of the proteins were under an OR of 1.10, with an average effect size of 1.09. Considering the observed prevalence rate of pathologic AD of 60% and α level of 0.05, the sample size required to detect an OR of 1.09 with 80% power was ≈4,400. The observed effect sizes for cortical Lewy bodies ranged between 1.05 and 1.64, with an average effect size of 1.23. The observed prevalence rate of cortical Lewy bodies was ≈15%, and the sample size required to detect an OR of 1.23 was ≈1,400. The estimated effect size for LATE-NC averaged across the 11 proteins was 1.19. With an observed prevalence rate of LATE-NC of 27%, the sample size required to detect an OR of 1.19 was ≈1,300. The average effect size for hippocampal sclerosis was ≈1.18. Because the prevalence rate of hippocampal sclerosis was relatively low (i.e., 8%), a larger sample (n= ≈3,900) was needed to detect an OR of 1.18 with 80% power.

Finally, we examined the statistical power for protein association with cerebral vascular conditions. The average effect sizes for cerebrovascular pathologies ranged between 1.10 and 1.13. eFigure 2, links.lww.com/WNL/B724 shows the sample sizes required to detect these ORs. To illustrate, our data suggest that the observed prevalence rate of chronic macroscopic infarcts was 32%, and a sample of 2,500 could achieve 80% power at an α level of 0.05 to detect an average effect size of 1.13.

Figure 2 Neuropathologic Correlates of Human Cortical Proteins in AD-Related Dementia



Neuropathologic correlates of human cortical proteins implicated in Alzheimer dementia are illustrated. Individual proteins are shown on the x-axis. Clinical and neuropathologic outcomes are shown on the y-axis. Red tile represents a positive association; blue tile represents a negative association. Size of black dot inside the tile signifies strength of an association. AD = Alzheimer disease; LATE-NC = limbic-predominant age-related TAR DNA binding protein *43* encephalopathy neuropathologic changes.

Discussion

By integrating the summary statistic from a large-scale GWAS meta-analysis of Alzheimer dementia with human brain proteomic and genetic data, we previously identified 11 novel cortical proteins implicated in Alzheimer dementia. Here, we extend the prior report by interrogating the neuropathologic correlates of these protein targets. Overall, we were able to confirm the association of these proteins with Alzheimer dementia. Five proteins were nominally associated with Alzheimer dementia proximate to death in our sample, and power calculation shows that with a sample size of ≈1,600, we will be able to detect all the protein signals except DOC2A and LACTB. These proteins were differentially associated with common neuropathologic indices. Furthermore, our results suggest that not all the protein associations with Alzheimer dementia work through these known pathologies. Implications of these findings are discussed.

Table 3 Cortical Proteins and Alzheimer Dementia Controlling for Neuropathologies

UniProtKB	Protein	Estimate	SE	*p* Value
P12821	ACE_HUMAN	0.238	0.119	0.046
Q9Y2V2	CHSP1_HUMAN	0.434	0.132	0.001
Q8NDH6	ICA1L_HUMAN	−0.211	0.121	0.082
Q12846	STX4_HUMAN	0.181	0.126	0.153

Abbreviation: SE = standard error.
Estimates are log odds ratios, and results are from separate logistic regression models controlled for demographics and neuropathologic indices associated with corresponding protein.

It has been increasingly recognized that Alzheimer dementia is not caused by AD alone. One study reported that by eliminating AD pathology, dementia prevalence among the oldest old could be reduced by 50%.[11] The estimate is lower in the ROSMAP cohort.[10] Furthermore, AD pathology explains only a part of the person-to-person variation in late-life

cognitive decline,[31] the clinical hallmark of Alzheimer dementia. Together, these findings suggest that neuropathologies underlying Alzheimer dementia are complex and that cortical proteins implicated in Alzheimer dementia do not necessarily have an AD pathogenesis. In this study, we did not find strong evidence for association of any of the 11 cortical protein targets with pathologic AD diagnosis. Power calculation showed that >4,000 samples are required to detect an average effect size observed in this study, suggesting that the impacts of these proteins on Alzheimer dementia lack an AD pathologic footprint.

Surprisingly, cortical protein associations with non-AD neurodegenerative conditions were stronger. On average, the effect sizes for cortical Lewy bodies, LATE-NC, and even hippocampal sclerosis were greater than that for pathologic AD. Multiple proteins were associated with cortical Lewy bodies; the CHSP1 protein was the strongest signal, and the association survived the correction for multiple testing. The protein is encoded by *CARHSP1* and has been implicated in oxidative stress response.[32] Separately, 4 proteins were nominally associated with LATE-NC, of which the CATH protein survived the correction for multiple testing. CATH, encoded by *CTSH*, is a lysosomal cysteine proteinase responsible for overall protein degradation in lysosomes. Due to a low prevalence of hippocampal sclerosis, we were unable to observe any protein signal related to the pathology. The associations with vascular conditions were weaker, and none survived the correction for multiple testing. Notably, however, the above-mentioned CHSP1 was also nominally associated with macroscopic infarcts and CATH was nominally associated with arteriolosclerosis. These results suggest that the neuropathologic correlates of CHSP1, and separately CATH, are not confined to a single pathologic index. The underlying biology that links these proteins to particular pathologies is unknown and warrants further investigation.

A more interesting protein signal is STX6, encoded by the syntaxin-6 gene. While higher protein expression was associated with a greater risk of Alzheimer dementia, we did not find a significant association with any of the neuropathologic indices investigated here. These results suggest that the protein association with Alzheimer dementia is likely attributable to factors beyond common neuropathologic indices. STX6 may represent a marker for less resilience such that it increases susceptibility of Alzheimer dementia independently of common neuropathologies. This result is consistent with our previous proteome-wide scan of cognitive resilience.[22] In that study, while the STX6 protein did not survive the correction for multiple testing, higher STX6 expression was indeed associated with faster cognitive decline after controlling for neuropathologic indices ($p < 0.006$). Notably, STX6 plays an important role in intracellular vesicle trafficking, including trafficking of key human disease-associated proteins.[33] In particular, studies have shown that STX6 regulates nerve growth factor–dependent neurite outgrowth, and overexpression of the first coiled-coil domain of STX6 inhibits the outgrowth.[34] Our result for STX6 suggests that some protein targets for Alzheimer dementia likely work through pathways including regulating neural circuit formation.

Strengths of this study include that brain samples came from community-dwelling old adults who were initially free of dementia and prospectively followed up until death. The follow-up rate (>90%) and autopsy rate (>85%) are high, reducing the selection bias. These older adults were deeply phenotyped. In particular, multiple neurodegenerative and cerebrovascular pathologies were assessed and recorded via a uniform structured procedure, which ensured measurement consistency. Advanced high-throughput proteomic sequencing method enables high-quality protein quantification from human cortical brain tissue. Limitations are noted. For the purpose of interpretation and comparison across different indices, dichotomous neuropathologic outcomes were used in the analyses. Therefore, the results do not inform on the protein association with disease severity. Furthermore, ROSMAP are voluntary cohorts and participants are predominantly White and older and have higher education. Findings should be interpreted with caution, and generalizability needs to be demonstrated.

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Patricia A. Boyle, PhD	Rush Alzheimer's Disease Center and Department of Psychiatry and Behavioral Sciences, Rush University Medical Center, Chicago, IL	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Aliza P. Wingo, MD	Division of Mental Health, Atlanta VA Medical Center, Decatur, GA; Department of Psychiatry, Emory University School of Medicine, Atlanta, GA	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data

Appendix *(continued)*

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