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RESEARCH ARTICLE

Unraveling sex differences in Alzheimer's disease and related endophenotypes with brain proteomes

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Abstract

INTRODUCTION: Sex differences exist in Alzheimer's disease (AD), but the underlying mechanisms remain unclear.

METHODS: We examined brain proteomes profiled from the dorsolateral prefrontal cortex of 770 donors (66.2% female).

RESULTS: Proteome-wide differential expression analysis in males and females jointly identified many significant proteins for AD dementia (n = 1228), amyloid beta (n = 1183), tangles (n = 1309), and global cognitive trajectory (n = 2325) at a false discovery rate of <0.05. Sex-stratified analyses also identified many proteins associated with AD or its endophenotypes. Finally, we found 10 proteins with significant sex-by-trait interactions, including one in AD clinical diagnosis (MARCKS), seven in cognitive trajectories (TOGARAM1, PLCD3, SLC22A5, MTFR1L, DCUN1D5, S100A12, and TRIM46), and two in cerebral pathologies (PANK4 and SOS1).

DISCUSSION: The 10 proteins with sex interaction in AD cover a range of functions likely relevant for AD pathogenesis, including estrogen response, inflammation, and mitochondrial biology, and their specific roles in AD ought to be studied. Future work should test their potential as sex-specific AD biomarkers.

KEYWORDS
Alzheimer's disease, brain proteomes, endophenotype, estrogen, sex difference

Aliza P. Wingo and Thomas S. Wingo contributed equally to this work.

Highlights

- At the phenotypic level, we found sex differences in baseline cognitive performance, cognitive trajectories, and AD hallmark pathologies.
- Proteome-wide differential expression analyses identified many brain proteins associated with AD and its endophenotypes in either sex alone or when considered together.
- We found 10 brain proteins with significant sex interactions in AD and its endophenotypes, which could be investigated as potential sex-specific biomarkers of AD.

1 | BACKGROUND

There is a long-standing interest in sex differences in Alzheimer's disease (AD). Females account for two-thirds of AD patients and experience a higher lifetime risk of developing AD compared to males.[1] One possible reason for this disparity is that females generally live longer than males, and age is the strongest risk factor for AD.[1] Epidemiological studies assessing AD incidence by sex show geographical and sociocultural variations. Studies conducted in Europe[2–4] and Asia[5,6] suggest a higher incidence in females at older ages, whereas studies from North and South America have not reported a significant sex difference in the incidence of AD.[7–11] Despite the mixed findings from these incidence studies, sex differences have been observed in the progression, diagnosis, and clinical presentations of AD.[12–14]

Multiple transcriptomic studies have identified potential molecular mechanisms underlying sex differences in AD. For instance, estrogen-related receptor beta was found to be differentially expressed between male and female AD across four brain regions.[15] Expression of the estrogen receptor had stronger associations with AD cognitive and pathologic traits in females than males.[16] Female-specific pathways were found to be enriched in immune response,[17–19] insulin signaling,[20,21] and tau phosphorylation,[22] while male-specific pathways were linked to nicotine addiction, adipocytokine signaling, and alcoholism.[20]

Compared to the transcriptomic studies, proteome-wide sex differences in AD remain largely understudied. An integrated proteomic and metabolomic study of 43 hippocampal samples identified significant sex differences in insulin signaling and serine metabolism.[23] Our prior work examined the effect of biological sex on protein expression and its genetic regulation in human brain proteomes.[24] However, proteome-wide sex differences in AD and related endophenotypes have not been explored.

Here, we performed sex-aware proteome-wide differential expression analyses in AD and its endophenotypes using deep brain proteomes generated from the dorsal lateral prefrontal cortex (DLPFC) of 770 donors. We first examined sex differences in AD clinical diagnosis, cognitive trajectories, AD hallmark pathologies, age-related pathologies, and brain protein expression. Then we conducted joint and sex-stratified analyses to comprehensively identify proteins associated with AD and its endophenotypes. Lastly, we tested significant proteins from joint and sex-stratified analyses for sex-by-trait interactions. While we find many brain proteins associated with AD and its endophenotypes in either sex alone or when considered together, only a limited number showed evidence of sex-biased disease associations. These proteins have established links with estrogen responses and, although modest, highlight important biology relevant to the effect of sex in AD that will likely be more completely revealed by studies using larger sample sizes.

2 | METHODS

2.1 | Study participants

Participants were recruited by the Religious Orders Study (ROS) or the Rush Memory and Aging Project (MAP), two ongoing longitudinal cohort studies of aging and dementia approved by an Institutional Review Board of Rush University Medical Center.[25,26] Both studies recruit older adults who enroll without known dementia and agree to annual clinical evaluation and brain donation at death. All participants signed an informed consent, the Anatomical Gift Act, and a repository consent to allow their study data to be repurposed. This study included 770 participants and their cognitive diagnosis at the time of death consisted of 33.4% no cognitive impairment (NCI), 26.9% mild cognitive impairment (MCI), and 39.7% AD dementia. The deep brain proteome of these participants was generated from the DLPFC. The DLPFC controls working memory and executive function and is a key neocortical region affected in AD.[27,28]

2.2 | Clinical traits and cerebral pathologies

2.2.1 | Clinical diagnosis

The clinical diagnosis of cognitive status was made at each assessment based on a three-stage process, including computer scoring of cognitive tests, clinical judgment by a neuropsychologist, and final diagnostic classification by a clinician.[29] For AD dementia, the diagnosis

followed the criteria set by the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). It requires evidence of a significant cognitive decline with impairment in memory and at least one other cognitive domain.[29] MCI was diagnosed in individuals who were judged to have cognitive impairment but did not meet the criteria for dementia.[30] Individuals without AD dementia or MCI were classified as having no cognitive impairment. A final cognitive diagnosis is made at the time of death after review of all data blinded to neuropathology. We included this final cognitive diagnosis in our analysis.

2.2.2 | Cognitive performance at baseline and cognitive trajectory

We assessed five different cognitive domains at annual intervals with a battery of cognitive tests as previously described.[31] Raw scores from each cognitive test were converted to z-scores using the baseline mean and standard deviation of the entire cohort. These z-scores were then averaged to create summary scores for each cognitive ability. The global cognitive performance was computed by averaging the summary scores of each cognitive domain.[31]

Cognitive trajectories refer to the estimated person-specific rate of change in cognitive performance over time. The rate of cognitive change is the random slope from a linear mixed-effects model with the annual cognitive performance as the longitudinal outcome, adjusting for age at recruitment, sex, and years of education.[32,33]

2.2.3 | Cerebral pathologies

Our analysis included AD hallmark pathology (amyloid beta [Aβ], paired helical filaments of tau protein [PHFtau] tangles, global AD pathology), and some co-occurring age-related neuropathologic conditions such as gross infarcts, microinfarcts, arteriolosclerosis, cerebral amyloid angiopathy (CAA), Lewy bodies, hippocampal sclerosis, and limbic-predominant age-related TAR DNA binding protein 43 (TDP-43) encephalopathy neuropathologic changes (LATE-NC). Aβ and tangles were measured in eight cortical regions by molecularly specific immunohistochemistry, and a composite score was generated for each subject using computer-assisted sampling.[34] Global AD pathology is a summary measure of neuritic plaques, diffuse plaques, and neurofibrillary tangles in five cortical regions determined by modified Bielschowsky silver staining.[35] We took the square root of the Aβ, PHF-tau tangle density, and global AD pathology scores to approximate a normal distribution.

Four cerebrovascular conditions were included in this study. Gross infarcts were identified through gross examination, and microinfarcts were assessed using H&E-stained sections from at least nine brain regions.[36] Both gross infarcts and microinfarcts were treated as binary variables (present vs absent) in our analysis. Arteriolosclerosis was assessed in the vessels of the anterior basal ganglia, and CAA was evaluated in four neocortical regions using immunostaining for Aβ deposition.[37] Severity scores for both arteriolosclerosis and CAA were treated as continuous variables in our analysis.

Lewy bodies were assessed using α-synuclein immunostaining, as previously described.[38] In this study, we used a dichotomous variable to indicate the presence or absence of neocortical-type Lewy bodies.[38] Hippocampal sclerosis was defined by the presence or absence of neuronal loss and gliosis in the CA1 region and/or subiculum.[39] LATE-NC was classified into four stages: stage 0 (absence of TDP-43), stage 1 (presence of TDP-43 in the amygdala), stage 2 (extension of TDP-43 to the entorhinal cortex and hippocampus), and stage 3 (extension of TDP-43 to the neocortex).[40] LATE-NC was treated as a binary variable (stage 0/1 vs stage 2/3) in our analysis.

2.2.4 | Other variables

The presence of diabetes mellitus and hypertension in past medical history or during any follow-up visit was included as covariates in our statistical models. Apolipoprotein E (*APOE*) genotyping was

RESEARCH-IN-CONTEXT

1. **Systematic review**: To find human brain omics studies examining the role of sex differences in AD, we searched PubMed with keywords "sex differences," "differential expression," "omics," "aging," and "Alzheimer's disease." Multiple transcriptomic studies have identified potential molecular mechanisms underlying sex differences in AD, ranging from immune response and insulin signaling to estrogen-related pathways and adipocytokine signaling. On the other hand, proteome-wide sex differences in AD remain largely understudied, with only one study identified using tissue from 43 donors that found differences in insulin signaling and serine metabolism. Our prior work examined the effect of biological sex on protein expression and its genetic regulation in human brain proteomes; however, proteome-wide sex differences in AD and related endophenotypes have not been explored.
2. **Interpretation**: Our study brings new insight into the role of sex in AD by identifying brain proteins associated with AD, cognitive trajectories, and AD-related pathologies that differ by sex. The identified proteins covered a range of functions that are likely relevant to AD pathogenesis, although no single unifying pathway was identified.
3. **Future Directions**: Testing the feasibility of the identified proteins as sex-specific AD biomarkers in CSF or blood and larger brain proteomic datasets in diverse populations is likely to further expand our understanding of sex differences in AD.

extracted from high throughput sequencing to assign *APOE* ε2, ε3, and ε4 genotypes using rs429358 and rs7412 alleles.[41,42]

2.3 | Proteomic sequencing and quality control

Methods for proteomic sequencing were described in detail previously.[43] In brief, each sample was homogenized and digested to obtain peptides, followed by isobaric tandem mass tag (TMT) peptide labeling and high pH fractionation. Fractions were then analyzed by liquid chromatography-mass spectrometry, and the resulting spectra were searched against the human UniProt database. Peptides were grouped using parsimony, and unique peptides were used for protein-level quantitation.

Quality control of the proteomic sequencing followed the same approach as in our previous studies.[24,44] Proteins with TMT abundance values in at least 30% of samples were included in our analyses. To normalize the raw data, each protein's abundance was first divided by the sum of abundance values of all the proteins profiled for that sample, followed by the log2 transformation. Principal component analysis was used to remove samples with greater than four standard deviations from the mean of either the first or second principal component. We used linear regression to regress out the effect of protein sequencing batch and *post mortem* interval. The resulting residual protein abundance was used for subsequent analyses.

2.4 | Statistical analysis

First, we systematically explored phenotypic differences by sex for AD and its endophenotypes. A chi-squared test was used to compare sex differences in AD cognitive diagnosis. Linear regression was used to compare baseline cognitive abilities between females and males adjusting for education and age at baseline. To compare sex differences in the rates of cognitive change over time, a linear mixed model was used to test for sex-by-time interaction with annual cognitive performance as the longitudinal outcome. The model included time (follow-up year), sex, education, age at baseline, sex-by-time interaction, education-by-time interaction, and age-by-time interaction as fixed effects, while allowing the slope and intercept to vary as random effects. To assess sex differences in brain pathologies, linear regression was used to compare continuous measures of AD pathologies (e.g., Aβ, tangles), and logistic regression was used to compare categorical pathologic measures (e.g., Lewy bodies) with adjustment for education and age at death. For the presence of diabetes and hypertension, we used logistic regression adjusting for education and age at baseline. The age variables adjusted in these models were based on the timing of the outcome assessment. For variables measured at the time of recruitment, we adjusted for the age at baseline, while for *post mortem* characteristics, we adjusted for the age at death.

The surrogate variable analysis (SVA) method in the sva R package (version 3.52.0)[45] was used to detect potentially hidden variables that may influence brain protein expression. For each studied trait, surrogate variables (SVs) were estimated based on the protein matrix while protecting the effects of sex, the trait, and the sex-by-trait interaction term. Significant SVs were then adjusted for as covariates in relation to protein expression.

To identify proteins differentially expressed between females and males, we performed linear regression with protein abundance as the dependent variable (Y) and sex as the independent variable and covaried for age at death, education, cognitive diagnosis, and SVs. Similarly, joint and sex-stratified analyses were performed with protein abundance as the dependent variable (Y) and AD or its endophenotype as the independent variable and adjusted for sex (in the joint analysis), age at death, education, and SVs. Significant proteins from the joint and sex-stratified analyses were tested for sex-by-trait interaction and covaried for age, education, and SVs. The rationale for testing sex-by-trait interactions was to determine whether the relationship between protein expression and AD traits differs by sex.

Functional enrichment of differentially expressed proteins was assessed using the clusterProfiler R package (version 4.13.4).[46] All proteins that passed the quality control (n = 10,030) were used as the background set. The z-score was used to determine overrepresentation of ontologies and one-sided Fisher's exact test (Benjamini–Hochberg false discover rate [FDR] corrected) was used to assess the significance of the z-score. Gene Ontology terms were considered significant at an adjusted p value of less than 0.05.

To determine the consistency between proteins associated with each trait, we estimated the replication rate, $\pi 1$, between the sex-stratified analyses. $\pi 1$ estimates the fraction of true signals, not just significance, which makes it less dependent on sample size and power[47] and helpful when the two analyses have different sample sizes (i.e., female dataset n = 510, male dataset n = 260). Significant proteins in the larger discovery (female) dataset at FDR < 0.05 were selected from the replication (male) dataset to estimate the $\pi 1$ statistics using the qvalue package (version 2.36.0).[48] All analyses were performed in R (version 4.4.0).

3 | RESULTS

3.1 | Comparing AD and its endophenotypes between females and males

A total of 770 ROS/MAP participants (66.2% female) having deep brain proteomic profiles were included in this study. Their clinical and *post mortem* characteristics are detailed in Table 1. All participants were of European ancestry, with an average age of 89.4 at death and an average of 15.9 years of education. Compared to male participants, female participants had an older age at death (β [SE] = 2.8 [0.5]; $p < 0.001$) and received fewer years of education (β [SE] = −1.4 [0.3]; $p < 0.001$). The final cognitive diagnosis for these participants consisted of 39.7% AD dementia, 26.9% MCI, and 33.4% NCI. There were no significant sex differences in cognitive diagnosis (p = 0.41) and *APOE* ε4 status (p = 0.90).

TABLE 1 Participant characteristics.

Characteristic	All	Female	Male	*p* value[a]
No. participants (%)	770	510 (66.2%)	260 (33.8%)	–
Age at baseline, mean (SD), years	81.0 (6.69)	81.7 (6.40)	79.6 (7.03)	<0.001
Age at death, mean (SD), years	89.4 (6.45)	90.4 (6.18)	87.6 (6.60)	<0.001
Years of education, mean (SD), years	15.9 (3.55)	15.4 (3.24)	16.8 (3.93)	<0.001
Follow-up duration, mean (SD), years	7.95 (4.45)	8.00 (4.35)	7.85 (4.65)	0.251
Cognitive diagnosis at death				
NCI	257 (33.4%)	164 (32.2%)	93 (35.8%)	0.41
MCI	207 (26.9%)	135 (26.5%)	72 (27.7%)	
AD	306 (39.7%)	211 (41.4%)	95 (36.5%)	
APOE ε4 carriers	186 (24.2%)	122 (23.9%)	64 (24.6%)	0.903
Cognitive measures at baseline, mean (SD)				
Global cognition	−0.112 (0.61)	−0.127 (0.57)	−0.083 (0.67)	0.157
Episodic memory	−0.121 (0.78)	−0.103 (0.76)	−0.157 (0.80)	0.002
Semantic memory	−0.071 (0.71)	−0.095 (0.68)	−0.024 (0.76)	0.723
Working memory	−0.029 (0.79)	−0.049 (0.78)	0.009 (0.82)	0.466
Visuospatial ability	−0.092 (0.82)	−0.230 (0.76)	0.176 (0.88)	<0.001
Perceptual speed	−0.203 (0.82)	−0.195 (0.79)	−0.218 (0.87)	0.024
Global AD pathology score, mean (SD)	0.748 (0.64)	0.816 (0.66)	0.613 (0.59)	0.005
Amyloid beta score, mean (SD)	4.53 (4.45)	4.82 (4.54)	3.98 (4.22)	0.113
Neurofibrillary tangle score, mean (SD)	6.28 (7.00)	7.02 (7.01)	4.84 (6.76)	<0.001
Arteriolosclerosis, moderate/severe	257 (33.4%)	183 (35.9%)	74 (28.5%)	0.043
Cerebral amyloid angiopathy, moderate/severe	259 (33.7%)	177 (34.7%)	82 (31.5%)	0.484
Chronic gross infarcts, present	261 (33.9%)	175 (34.3%)	86 (33.1%)	0.453
Chronic microinfarcts, present	214 (27.8%)	147 (28.8%)	67 (25.8%)	0.973
Lewy bodies, neocortical type	91 (11.8%)	52 (10.2%)	39 (15.0%)	0.102
LATE-NC, stage2/3	229 (29.7%)	170 (33.3%)	59 (22.7%)	0.111
Hippocampal sclerosis, present	53 (6.9%)	39 (7.6%)	14 (5.4%)	0.678
Diabetes, present	153 (19.9%)	82 (16.1%)	71 (27.3%)	0.006
Hypertension, present	520 (67.5%)	366 (71.8%)	154 (59.2%)	0.002

[a]*p* values are from regression models comparing phenotypic differences by sex.
Abbreviations: AD, Alzheimer's disease; LATE-NC, limbic-predominant age-related TAR DNA binding protein 43 encephalopathy neuropathologic changes; MCI, mild cognitive impairment; NCI, no cognitive impairment; SD, standard deviation.

The median follow-up duration for all participants was 7.9 years prior to death. At baseline, female participants showed better performance in episodic memory (β [SE] = 0.17 [0.06]; $p = 0.002$) and perceptual speed (β [SE] = 0.14 [0.06]; $p = 0.024$), while male participants showed better baseline visuospatial ability (β [SE] = 0.35 [0.06]; $p < 0.001$) after adjusting for age and education (Table S1). The linear mixed model and time-by-sex interaction term were used to assess sex differences in rates of cognitive change over time. Both female and male participants displayed significant declines in global cognition and different cognitive domains over time. Compared to male participants, female participants had lower cognitive scores at each assessment timepoint (i.e., negative time-by-sex interaction term), indicating steeper rates of decline in global cognition and cognitive abilities (Table S1).

When comparing AD hallmark pathology by sex, female participants had a higher global AD pathology score (β [SE] = 0.11 [0.03]; $p < 0.001$) and a higher neurofibrillary tangle score (β [SE] = 0.38 [0.09]; $p < 0.001$) after adjusting for education and age at death. Although females had a higher Aβ load than males, the difference was not statistically significant after controlling for education and age at death ($p = 0.113$). For age-related pathologies, females showed more severe arteriolosclerosis than males (β [SE] = 0.15 [0.07]; $p = 0.043$) while no significant sex differences were found in other age-related neuropathologic conditions. In summary, these data suggest the pres-

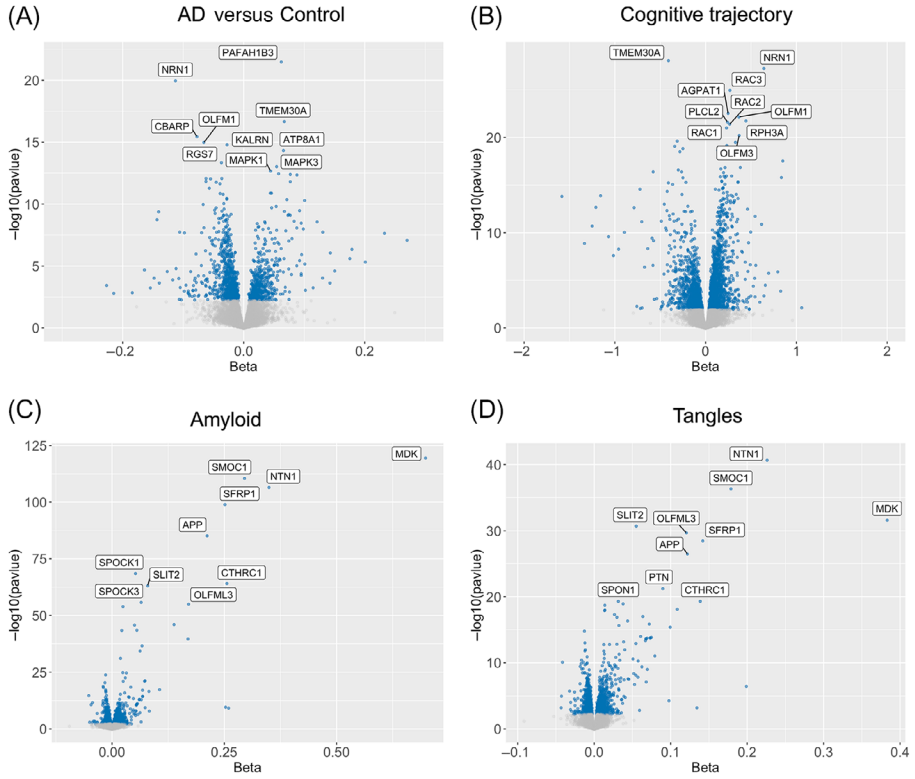

FIGURE 1 Differential protein expression in AD endophenotypes for (A) clinical diagnosis, (B) rates of change of global cognitive performance, (C) amyloid, and (D) tangles. Each volcano plot presents the beta coefficients and $-\log_{10}(p$ values) from linear regression of each protein and phenotypic outcome adjusting for demographics and surrogate variables. Blue points indicate significant proteins at a false discovery rate of <0.05. The top 10 differentially expressed proteins are labeled with their gene symbols in white boxes. Beta coefficients and statistics for all proteins are provided in Supplementary Tables (Tables S3 and S5, Tables S12 and S13).

ence of sex differences in baseline cognitive performance, cognitive trajectories, and AD hallmark pathologies.

3.2 | Sex difference in brain protein expression

Next, we sought to identify proteins differentially expressed between females and males. After quality control, 10,030 proteins were considered in our study. We regressed out the effects of the protein sequencing batch and *post mortem* interval and used the residual protein abundance for further proteome-wide differential analyses. To identify sex-differentiated proteins, we first used surrogate variable analysis (SVA) to estimate hidden technical or biological factors that may influence brain protein expression such as cell-type heterogeneity. Then we fitted a linear regression model with protein abundance as the dependent variable (Y) and sex as the independent variable and adjusted for education, age at death, cognitive diagnosis, and surrogate variables. Among the 10,030 measured proteins, 1467 showed sex-differentiated expression at the FDR cutoff of 5%. Of these, 41% had higher expression in males and 59% had higher expression in females (Table S2). As expected, the top differentially expressed proteins were encoded by genes on the X chromosome. These findings are in line with our prior work showing robust differences in brain protein expression by sex.[24]

3.3 | Proteome-wide differential expression analysis of AD

To comprehensively identify significant proteins associated with AD and its endophenotypes, we performed proteome-wide differential expression analysis jointly in females and males and in each sex separately. The rationale for conducting both joint and sex-stratified analyses is that a joint analysis can maximize statistical power to detect proteins relevant to the disease, while a sex-stratified analysis can detect proteins with opposite effects in different sex groups. For each phenotypic outcome, SVA was used to infer hidden technical or biological factors, such as cell-type heterogeneity, that may influence brain protein levels. Then we fitted a linear regression model with protein expression as the dependent variable (Y) and AD or its endophenotypes as the independent variable and adjusted for sex (in the joint analysis), age at death, education, and surrogate variables.

In the joint analysis between AD and control, 1228 proteins were differentially expressed with FDR < 0.05 (Table S3). As shown in Figure 1A, proteins with positive betas were more abundant, and conversely, those with negative betas were less abundant in AD. The upregulated proteins in AD were enriched in processes related to intermediate filament, cell chemotaxis, and phospholipid transport (Figure S1, Table S4). The downregulated proteins in AD were enriched for synaptic signaling, axon development, and actin filament organization

(Figure S1, Table S4). Joint analysis in rates of change of global cognition revealed 2325 significant proteins (at FDR < 5%, Table S5). Among these proteins, 59% had higher abundance (i.e., positive betas) in cognitive stability, while 41% had lower abundance (i.e., negative betas) in cognitive stability (Figure 1B). The upregulated proteins associated with cognitive stability were enriched for synapse organization, oxidative phosphorylation, and actin filament organization, while the downregulated proteins were enriched for processes related to intermediate filaments and endosomal transport (Figure S1, Table S6). NRN1 was the top protein upregulated in cognitive stability, consistent with prior studies indicating its association with a slower rate of cognitive decline independent of AD and related dementia pathologies.[49,50] Joint analysis in different cognitive domains also revealed many differentially expressed proteins, with 1976 for episodic memory, 1576 for working memory, 1501 for semantic memory, 461 for perceptual speed, and four for visuospatial ability (Figure S2, Table S7-S11).

In the joint analysis of AD hallmark pathologies, 1183 proteins were found to be associated with Aβ density, 1309 with tangle density, and 1924 with global AD pathology (Table S12-S14). The higher-abundance proteins in Aβ and tangles were both enriched for intermediate filament-based process, response to wounding, and extracellular matrix binding, while the lower-abundance proteins in Aβ and tangles were enriched for axon development and synaptic transmission (Figure S1, Table S15-S16). As depicted in Figure 1C and D, MDK, SMOC1, and NTN1 were the top proteins positively associated with both Aβ and tangles. These proteins have been shown to colocalize with Aβ plaque and are promising biomarkers associated with AD pathology.[43,51,52] Fewer proteins were found to be associated with age-related pathologies: 98 were associated with Lewy bodies, 67 with CAA, one with microinfarcts, and no associations observed with other neuropathologic conditions (Figure S2, Table S17-S23).

After finding robust protein expression differences in the joint analyses for AD dementia, cognitive traits, and AD hallmark pathologies, we next performed sex-stratified analyses. We first examined AD dementia and its related cognitive endophenotypes (i.e., cognitive trajectory). Sex-stratified analyses between AD and control yielded 869 significant proteins in females and 68 in males at a FDR threshold of 5%, with 35 proteins significant in both analyses and displaying concordant direction of associations (Figure 2A, Table S3). When using the female group as the discovery dataset, the estimated replication rate (π_1 statistics) for the male group was 56%, indicating that 56% of significant proteins identified in the female-stratified analysis were replicated in the male-stratified analysis. In sex-stratified analyses of the rates of change of global cognition, 1579 proteins were found to be differentially expressed in females and 538 proteins were differentially expressed in males with FDR < 0.05 (Figure 2B, Table S5). Notably, 393 proteins were significant in both stratified analyses and exhibited the same direction of associations. When using the female group as the discovery dataset, the estimated replication rate for the male group was 81%. The replication rates for different cognitive abilities were 77% for episodic memory, 84% for working memory, and 56% for semantic memory.

Next, we performed sex-stratified analysis of hallmark AD pathologies. Female-stratified analysis yielded 745 proteins associated with Aβ and 592 associated with tangles, while male-stratified analyses yielded 232 significant proteins in Aβ and 80 significant proteins in tangles (Figure 2C, Figure 2D, Table S12-S13). The number of proteins significant in both sex-stratified analyses was 152 for Aβ and 45 for tangles. When the female group was used as the discovery dataset, the replication rates for Aβ and tangles were 63% and 67%. Sex-stratified analyses of global AD pathology identified 1522 significant proteins in females and 231 in males at a FDR threshold of 5% (Figure S3, Table S14). The estimated replication rate for the male group was 50%. Regarding age-related pathologies, the female-stratified analysis identified 33 significant proteins for Lewy bodies and 46 for CAA, while the male-stratified analysis identified two proteins for Lewy bodies, nine for CAA, and one for hippocampal sclerosis (Figure S3, Tables S17 to S23).

Collectively, the joint and sex-stratified differential analyses indicate robust protein expression differences for AD and its endophenotypes. The replication rates observed in sex-stratified analyses (mean: 67%; range: 50% to 84%) suggest moderate to high consistency in the differentially expressed proteins associated with AD and related endophenotypes between the sexes.xxx

3.4 | Sex-by-trait interaction in AD

To investigate whether sex moderates the relationship between proteins and AD or its endophenotypes, we tested significant proteins in joint and sex-stratified analyses for sex-by-trait interaction adjusting for age at death, education, and surrogate variables. We first examined sex-by-trait interaction for AD dementia. In the joint and sex-stratified analysis between AD and control, a sum of 1364 proteins passed an FDR threshold of 5%. Among these significant proteins, one protein, MARCKS, had a significant sex-by-trait interaction at FDR < 0.05 (Table 2, Figure S4). MARCKS is a cellular substrate for protein kinase C (PKC) (Figure S5) and the phosphorylation level of MARCKS is associated with AD.[53,54]

Next, we examined sex-by-trait interaction for cognitive trajectories. Seven proteins were found to have significant sex-by-trait interactions with FDR < 0.05 (Table 2, Figure S4). Specifically, TOGARAM1, PLCD3, and SLC22A5 showed a significant sex interaction with the rates of change of episodic memory; MTFR1L displayed a significant sex interaction with the rates of change of working memory; and DCUN1D5, S100A12, and TRIM46 showed a significant sex interaction with the rates of change of semantic memory. Among these proteins, TOGARAM1 and TRIM46 are associated with proper microtubule functions,[55,56] and MTFR1L is associated with mitochondrial fusion processes.[57] DCUN1D5 is involved in the neddylation pathway that regulates protein homeostasis,[58] and S100A12 is a pro-inflammatory mediator that modulates neuroinflammation.[59] Both PLCD3 and SLC22A5 are associated with estrogen-related signaling pathways (Figure S5), with PLCD3 being a phospholipase C (PLC) isozyme activated by G protein-coupled estrogen receptor (GPER)[60,61]

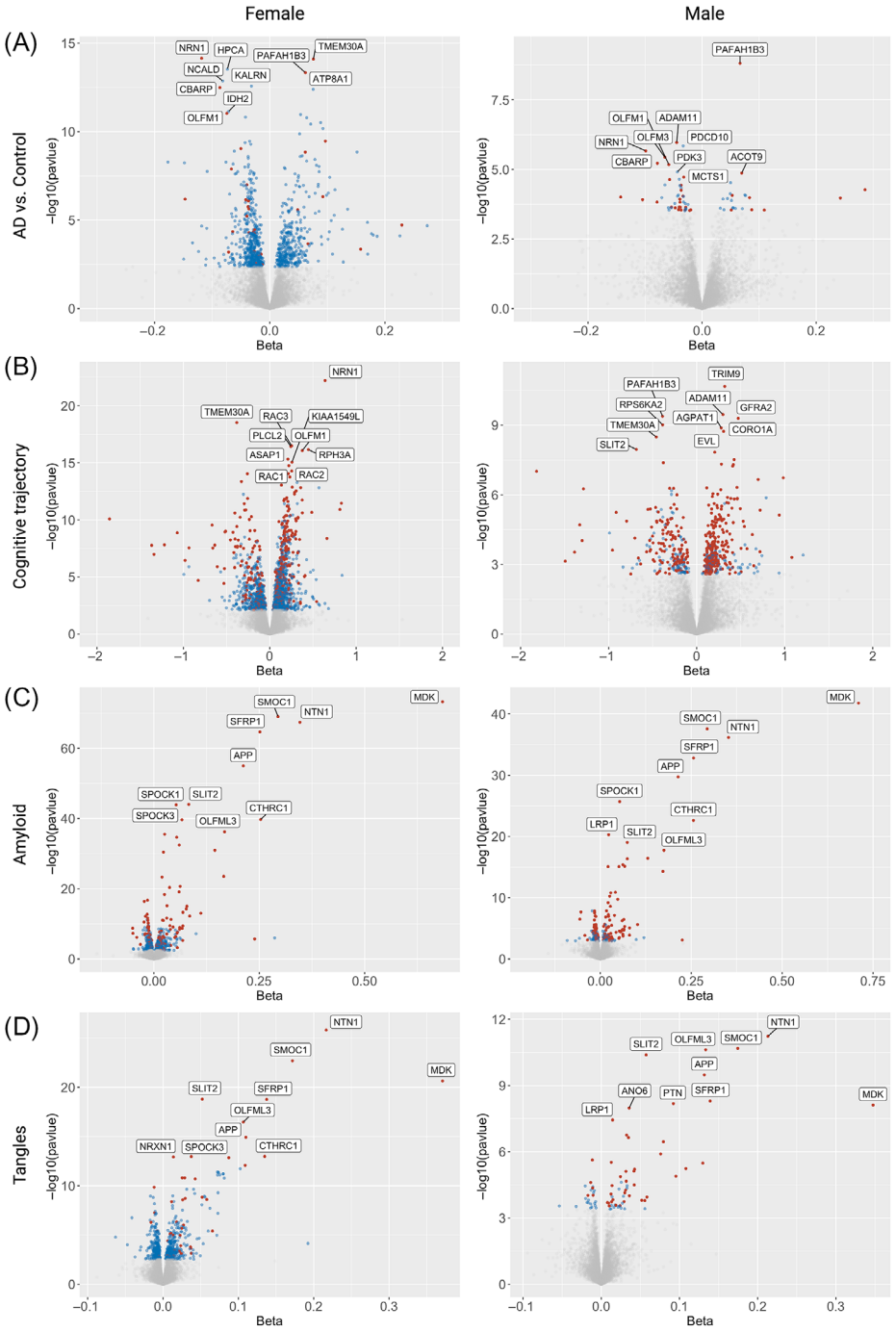

FIGURE 2 Sex-stratified differential protein expression in AD endophenotypes for (A) clinical diagnosis, (B) rates of change of global cognitive function, (C) amyloid, and (D) tangles. Each volcano plot presents the beta coefficients and $-\log_{10}(p$ values) from linear regression of each protein and phenotypic outcome adjusting for demographics and surrogate variables. Red points indicate proteins significant in both female and male-stratified analyses at FDR < 0.05 while blue points indicate proteins significant in one sex at a false discovery rate of <0.05. Top differentially expressed proteins are labeled with white boxes. Beta coefficients and statistics for all proteins are provided in Supplementary Tables (Tables S3 and S5, Tables S12 and S13).

and SLC22A5 being a carnitine transport protein transcriptionally regulated by estrogen receptors.[62]

In the sex-by-trait interaction analyses for cerebral pathologies, two proteins passed the 5% FDR threshold for global AD pathology and CAA (Table 2, Figure S4). Of these two proteins, PANK4 showed a sex-biased association with global AD pathology and is one of the pantothenate kinase isozymes implicated in coenzyme A biosynthesis.[63] SOS1 showed a significant sex interaction with CAA and is a guanine nucleotide exchange factor that activates the RAS signaling cascade.[64]

TABLE 2 Sex-biased proteins in AD endophenotypes.

		Females		Males		Sex interaction	
Trait	Protein	Beta	FDR	Beta	FDR	Beta	FDR
AD versus control	MARCKS	−0.05	0.0004	0.02	0.463	0.08	0.011
Trajectory of episodic memory	TOGARAM1	−0.10	0.532	0.81	0.005	0.80	0.008
	PLCD3	−0.01	0.934	−0.29	0.0005	−0.28	0.023
	SLC22A5	0.06	0.599	−0.35	0.032	−0.44	0.028
Trajectory of semantic memory	DCUN1D5	−0.31	0.345	1.51	0.006	1.57	0.030
	S100A12	−0.22	0.224	0.67	0.024	0.88	0.030
	TRIM46	−0.03	0.566	−0.25	0.0003	−0.21	0.030
Trajectory of working memory	MTFR1L	0.004	0.976	−0.44	0.001	−0.44	0.009
Global AD pathology	PANK4	−0.02	0.003	0.02	0.206	0.04	0.030
Cerebral amyloid angiopathy	SOS1	−0.007	0.038	0.004	0.885	0.01	0.039

Abbreviations: AD, Alzheimer's disease; FDR, false discovery rate.

To test whether comorbid medical conditions confound the moderating effects of sex on these phenotypic outcomes, we performed a sensitivity analysis by adjusting for the presence of diabetes and hypertension, both of which showed significant sex differences in our dataset (Table 1). All the sex-biased proteins remained significant in the sex-by-trait interaction analyses (Table S24), further suggesting the robustness of our findings. Overall, our analysis identified some promising proteins and cellular processes with sex-biased association in AD and AD endophenotypes.

4 | DISCUSSION

While transcriptome-wide sex differences in AD have been characterized in multiple studies,[15–22] proteome-wide sex differences in AD remain underexplored. In this study, we included 770 ROS/MAP participants with brain proteomic profiles generated from the DLPFC and performed sex-aware proteome-wide differential expression analyses in AD and its endophenotypes. Notably, we identified some sex-biased proteins associated with AD and its endophenotypes in the brain. These proteins could be investigated in cerebrospinal fluid (CSF) as potential biomarkers for sex-specific risk or further tested in model systems. To place these findings in context, below we examine the role of sex on phenotypic outcomes in ROS/MAP and highlight the potential biological role of the proteins we identified to have sex-biased disease associations.

Our phenotypic analysis suggests the presence of sex differences in baseline cognitive performance and cognitive trajectories. Specifically, females outperformed males in baseline episodic memory and processing speed but experienced greater declines in cognitive performance over time. These findings align with previous studies suggesting that females generally have better verbal and episodic memory than males,[65–67] though this memory advantage seems to decrease as females progress toward a diagnosis of cognitive impairment. Research has shown that females with MCI or AD dementia shows greater cognitive decline and are outperformed by males in several cognitive domains.[68,69] Moreover, *post mortem* studies indicate that females have lower resistance to AD pathologies.[70,71] Our study and others support the notion that the initial memory advantage in females appears to diminish over time with the loss of coping mechanisms in pathological aging.

Interestingly, some sex-biased proteins we identified are involved in estrogen-related pathways, including PLCD3, MARCKS, and SLC22A5 (Figure S5). PLCD3 is a phospholipase C (PLC) isozyme activated by G protein-coupled estrogen receptor (GPER) and is involved in phosphoinositide turnover and protein kinase C (PKC) activation.[60,61] PLCD is highly expressed in the brain and has been associated with AD and neurodegenerative diseases.[72] MARCKS is the main substrate for PKC, and the phosphorylation level of MARCKS is associated with Aβ biogenesis and neuron degeneration.[53,54,73] SLC22A5 is a carnitine transporter involved in neurotransmitter synthesis, and expression of SLC22A5 is regulated by estrogen receptors.[62] PKC also regulates the activity and translocation of SLC22A5.[74] Notably, PKC is central to the molecular pathways of all these proteins. Studies have shown that PKC is one of the key kinases activated in AD, and PKC substrates make up over half of the core molecules in AD.[75,76] This suggests that different PKC activity between females and males may account for some of the sex differences in AD. Our results corroborate previous studies that highlighted a sex-specific association between AD and estrogen receptors.[15,16] Further functional studies are needed to elucidate the role of these sex-biased proteins in AD.

Several sex-biased proteins identified in our study are also involved in molecular processes that may contribute to AD pathogenesis. For instance, S100A12 is a pro-inflammatory protein localized within Aβ plaques.[59,77] SOS1 activates the RAS signaling pathway, and its expression level is elevated in the blood of AD patients.[78] Both MTFR1L and PANK4 are associated with mitochondrial metabolism, with MTFR1L implicated in mitochondrial fusion process[57] and PANK4 involved in coenzyme A biosynthesis.[63] Moreover, TRIM46 and TOGARAM1 are proteins that play roles in microtubule functions,[55,56] and DCUN1D5

regulates the protein neddylation pathway that has been linked to AD.[58] Although sex differences in immune response[17–19] and mitochondrial metabolism[79] have been reported in AD, other molecular processes identified here remain understudied. Given the important overlap between brain and CSF proteomes,[80–82] further studies could investigate these proteins as potential sex-specific biomarkers in AD.

Consistent with previous proteomic studies of AD brain tissue, our analysis identified robust protein expression differences in AD and AD hallmark pathology. When compared with a recent large-scale proteomics study across diverse racial and ethnic groups,[83] 58% of the significant proteins in AD dementia and 68% of the significant proteins in amyloid identified here overlapped with their findings and showed concordant directions of association. We also found a 60% π_1 replication rate with earlier studies ($n = 516$ DLPFC samples) for clinical diagnosis,[43] suggesting the replicability of prior results. In addition to AD and AD pathology, we also found differential expression in age-related pathologies such as CAA and Lewy body pathology. In CAA, some matrisome proteins (SMOC1, NTN1, MDK) were found to be significantly upregulated. These proteins are strongly associated with amyloid plaques and are considered hub proteins in both AsymAD and AD.[43,51,80] Similarly, the muscarinic cholinergic receptor CHRM3, which is involved in the acetylcholine pathway,[84] was significantly upregulated in both AD and Lewy body pathology. The presence of these shared proteins may suggest a close interaction between AD and co-occurring neuropathologic conditions.

While hundreds of proteins were differentially expressed between sexes or differentially expressed in AD and its endophenotypes, only a small number of them had significant sex-by-trait interactions. These results suggest that the effect size of sex-based interactions is likely small. A meaningful investigation of interactions may require a nearly 10-fold increase in sample size compared to that needed for detecting the main effects.[85] Limited findings from genome-wide[86] and transcriptome-wide[18,22] association studies in AD also suggest that the sample size is a critical consideration when examining sex interactions.

Our study has potential limitations that will require further exploration in future work. First, this is a cross-sectional study; therefore, causal relationship cannot be inferred between these proteins and AD. Second, a limited number of proteins showed significant sex-by-trait interactions in our analysis, which may imply a lack of statistical power. Using a meta-analysis approach to combine results from different cohorts may increase the power to detect sex-based interactions. Third, our study focused on tissue from the DLPFC, which was selected owing to the relatively lower burden of AD pathology and neuronal loss, even among individuals with advanced AD or advanced age at death of the participants. Notably, we expect to be best powered to detect differences in proteins from neurons and glia, the major cell types in the gray matter that are also highly relevant for AD. Lastly, our analysis was based on self-identified white participants owing to low statistical power in other groups, which may limit the generalizability of our findings.

In conclusion, our study identified many brain proteins associated with AD, cognitive trajectories, and AD-related pathologies. We identified sex-biased proteins associated with AD and its endophenotypes in the brain that could be investigated as potential biomarkers for sex-specific risk. Future studies to test these proteins and expand omics analysis into additional brain regions (e.g., hippocampus and entorhinal cortex), rare cell types (e.g., brain endothelial cells), and additional populations would enhance our understanding of the role of sex in AD.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

The Religious Orders Study or the Memory and Aging Project was approved by an Institutional Review Board of Rush University Medical Center. All participants provided written informed consent.

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REFERENCES

1. 2024 Alzheimer's Disease Facts and Figures. *Alzheimers Dement*, 2024. 20(5):3708-3821.
2. Fratiglioni L, Viitanen M, Von Strauss E, Tontodonati V, Herlitz A, Winblad B. Very old women at highest risk of dementia and Alzheimer's disease: incidence data from the Kungsholmen project, Stockholm. *Neurology*. 1997;48(1):132-138.
3. Ott A, Breteler MMB, Harskamp FV, Stijnen T, Hofman A. Incidence and risk of dementia. The Rotterdam Study. *Am J Epidemiol*. 1998;147(6):574-580.
4. Letenneur L, Gilleron V, Commenges D, Helmer C, Orgogozo JM, Dartigues JF. Are sex and educational level independent predictors of dementia and Alzheimer's disease? Incidence data from the PAQUID Project. *J Neurol Neurosurg Psychiatry*. 1999;66(2):177-183.
5. Yoshitake T, Kiyohara Y, Kato I, et al. Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly japanese population: the hisayama study. *Neurology*. 1995;45(6):1161-1168.
6. Liu CK, Lai CL, Tai CT, Lin RT, Yen YY, Howng SL. Incidence and subtypes of dementia in southern Taiwan: impact of Socio-demographic Factors. *Neurology*. 1998;50(6):1572-1579.
7. Rocca WA, Cha RH, Waring SC, Kokmen E. Incidence of dementia and Alzheimer's disease: a reanalysis of data from Rochester, Minnesota, 1975-1984. *Am J Epidemiol*. 1998;148(1):51-62.
8. Ganguli M, Dodge HH, Chen P, Belle S, Dekosky ST. Ten-year incidence of dementia in a rural elderly US community population: the MoVIES Project. *Neurology*. 2000;54(5):1109-1116.
9. Kawas C, Gray S, Brookmeyer R, Fozard J, Zonderman A. Age-specific incidence rates of Alzheimer's disease: the baltimore longitudinal study of aging. *Neurology*. 2000;54(11):2072-2077.

10. Hebert LE, Scherr PA, Mccann JJ, Beckett LA, Evans DA. Is the risk of developing Alzheimer's disease greater for women than for men?. *Am J Epidemiol*. 2001;153(2):132-136.
11. Kukull WA, Higdon R, Bowen JD, et al. Dementia and Alzheimer disease incidence: a prospective cohort study. *Arch Neurol*. 2002;59(11):1737-1746.
12. Mielke MM, Vemuri P, Rocca WA. Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences. *Clin Epidemiol*. 2014;6:37-48.
13. Nebel RA, Aggarwal NT, Barnes LL, et al. Understanding the impact of sex and gender in Alzheimer's disease: a call to action. *Alzheimers Dement*. 2018;14(9):1171-1183.
14. Ferretti MT, Iulita MF, Cavedo E, et al. Sex differences in Alzheimer disease—the gateway to precision medicine. *Nat Rev Neurol*. 2018;14(8):457-469.
15. Sun LL, Yang SL, Sun H, Li WD, Duan SR. Molecular differences in Alzheimer's disease between male and female patients determined by integrative network analysis. *J Cell Mol Med*. 2019;23(1):47-58.
16. Oveisgharan S, Yang J, Yu L, et al. Estrogen receptor genes, cognitive decline, and Alzheimer disease. *Neurology*. 2023;100(14):e1474-e1487.
17. Paranjpe MD, Belonwu S, Wang JK, et al. Sex-specific cross tissue meta-analysis identifies immune dysregulation in women with Alzheimer's disease. *Front Aging Neurosci*. 2021;13:735611.
18. Brooks LRK, Mias GI. Data-driven analysis of age, sex, and tissue effects on gene expression variability in Alzheimer's Disease. *Front Neurosci*. 2019;13:392.
19. Barber AJ, Del Genio CL, Swain AB, et al. Age, Sex and Alzheimer's Disease: a Longitudinal Study of 3xTg-AD mice reveals sex-specific disease trajectories and inflammatory responses mirrored in postmortem brains from Alzheimer's patients. *Alzheimers Res Ther*. 2024;16(1):134.
20. Santiago JA, Quinn JP, Potashkin JA. Sex-specific transcriptional rewiring in the brain of Alzheimer's disease patients. *Front Aging Neurosci*. 2022;14:1009368.
21. Santiago JA, Bottero V, Potashkin JA. Transcriptomic and network analysis highlight the association of diabetes at different stages of Alzheimer's disease. *Front Neurosci*. 2019;13:1273.
22. Cáceres A, González JR. Female-specific risk of Alzheimer's disease is associated with tau phosphorylation processes: a transcriptome-wide interaction analysis. *Neurobiol Aging*. 2020;96:104-108.
23. Mangold CA, Wronowski B, Du M, et al. Sexually divergent induction of microglial-associated neuroinflammation with hippocampal aging. *J Neuroinflammation*. 2017;14(1):141.
24. Wingo AP, Liu Y, Gerasimov ES, et al. Sex differences in brain protein expression and disease. *Nat Med*. 2023;29(9):2224-2232.
25. A Bennett D, A Schneider J, Arvanitakis Z, S Wilson R. Overview and findings from the religious orders study. *Curr Alzheimer Res*. 2012;9(6):628-645.
26. A Bennett D, A Schneider J, S Buchman A, L Barnes L, A Boyle P, S Wilson R. Overview and findings from the rush memory and aging project. *Curr Alzheimer Res*. 2012;9(6):646-663.
27. Blumenfeld RS, Ranganath C. Dorsolateral prefrontal cortex promotes long-term memory formation through its role in working memory organization. *J Neurosci*. 2006;26(3):916-925.
28. De Jager PL, Ma Y, Mccabe C, et al. A multi-omic atlas of the human frontal cortex for aging and Alzheimer's disease research. *Sci Data*. 2018;5:180142.
29. Bennett DA, Schneider JA, Aggarwal NT, et al. Decision rules guiding the clinical diagnosis of Alzheimer's disease in two community-based cohort studies compared to standard practice in a clinic-based cohort study. *Neuroepidemiology*. 2006;27(3):169-176.
30. Bennett DA, Wilson RS, Schneider JA, et al. Natural history of mild cognitive impairment in older persons. *Neurology*. 2002;59(2):198-205.
31. Wilson RS, Boyle PA, Yu L, et al. Temporal course and pathologic basis of unawareness of memory loss in dementia. *Neurology*. 2015;85(11):984-991.
32. De Jager PL, Shulman JM, Chibnik LB, et al. A genome-wide scan for common variants affecting the rate of age-related cognitive decline. *Neurobiol Aging*. 2012;33(5):1017 e1-15.
33. Wilson RS, Beckett LA, Barnes LL, et al. Individual differences in rates of change in cognitive abilities of older persons. *Psychol Aging*. 2002;17(2):179-193.
34. Wilson RS, Arnold SE, Schneider JA, Tang Y, Bennett DA. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *J Neurol Neurosurg Psychiatry*. 2007;78(1):30-35.
35. Bennett DA, Wilson RS, Schneider JA, et al. Apolipoprotein E epsilon4 Allele, AD pathology, and the clinical expression of Alzheimer's disease. *Neurology*. 2003;60(2):246-252.
36. Arvanitakis Z, Capuano AW, Leurgans SE, Bennett DA, Schneider JA. Relation of cerebral vessel disease to Alzheimer's disease dementia and cognitive function in elderly people: a cross-sectional study. *Lancet Neurol*. 2016;15(9):934-943.
37. Boyle PA, Yu L, Nag S, et al. Cerebral amyloid angiopathy and cognitive outcomes in community-based older persons. *Neurology*. 2015;85(22):1930-1936.
38. Schneider JA, Arvanitakis Z, Yu L, Boyle PA, Leurgans SE, Bennett DA. Cognitive impairment, decline and fluctuations in older community-dwelling subjects with Lewy Bodies. *Brain*. 2012;135(Pt 10):3005-3014.
39. Nag S, Yu L, Capuano AW, et al. Hippocampal Sclerosis and TDP-43 pathology in aging and Alzheimer disease. *Ann Neurol*. 2015;77(6):942-952.
40. Nag S, Yu L, Boyle PA, Leurgans SE, Bennett DA, Schneider JA. TDP-43 pathology in anterior temporal pole cortex in aging and Alzheimer's disease. *Acta Neuropathol Commun*. 2018;6(1):33.
41. Yu L, Lutz MW, Wilson RS, et al. TOMM40'523 variant and cognitive decline in older persons with APOE epsilon3/3 genotype. *Neurology*. 2017;88(7):661-668.
42. Ghebranious N, et al. Detection of ApoE E2, E3 and E4 Alleles Using MALDI-TOF mass spectrometry and the homogeneous mass-extend technology. *Nucleic Acids Res*. 2005;33(17):e149.
43. Johnson ECB, Carter EK, Dammer EB, et al. Large-scale deep multi-layer analysis of Alzheimer's disease brain reveals strong proteomic disease-related changes not observed at the RNA Level. *Nat Neurosci*. 2022;25(2):213-225.
44. Wingo TS, Liu Y, Gerasimov ES, et al. Shared mechanisms across the major psychiatric and neurodegenerative diseases. *Nat Commun*. 2022;13(1):4314.
45. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva Package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics*. 2012;28(6):882-883.
46. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284-287.
47. Storey JD. A direct approach to false discovery rates. *Journal of the Royal Statistical Society Series B-Statistical Methodology*. 2002;64:479-498.
48. Storey JD, Bass A, Dabney A, Robinson D. *qvalue: Q-value Estimation for False Discovery Rate Control*. R package version 2.36.0; 2024. http://github.com/jdstorey/qvalue. Available from.
49. Yu L, Tasaki S, Schneider JA, et al. Cortical Proteins associated with cognitive resilience in community-dwelling older persons. *JAMA Psychiatry*. 2020;77(11):1172-1180.
50. Zammit AR, Klein H-U, Yu L, et al. Proteome-wide analyses identified cortical proteins associated with resilience for varied cognitive abilities. *Neurology*. 2024;102(1):e207816.

51. Bai B, Wang X, Li Y, et al. Deep multilayer brain proteomics identifies molecular networks in Alzheimer's disease progression. *Neuron*. 2020;105(6):975-991 e7.
52. Zhou M, Haque RU, Dammer EB, et al. Targeted mass spectrometry to quantify brain-derived cerebrospinal fluid biomarkers in Alzheimer's disease. *Clin Proteomics*. 2020;17:19.
53. Chen Z, Zhang W, Selmi C, et al. The myristoylated alanine-rich C-kinase Substrates (MARCKS): a membrane-anchored mediator of the cell function. *Autoimmun Rev*. 2021;20(11):102942.
54. Fujita K, Motoki K, Tagawa K, et al. HMGB1, a pathogenic molecule that induces neurite degeneration via TLR4-MARCKS, is a potential therapeutic target for Alzheimer's Disease. *Sci Rep*. 2016;6:31895.
55. Latour BL, et al. Dysfunction of the Ciliary ARMC9/TOGARAM1 protein module causes Joubert Syndrome. *J Clin Invest*. 2020;130(8):4423-4439.
56. van Beuningen SFB, et al. TRIM46 controls neuronal polarity and axon specification by driving the formation of parallel microtubule arrays. *Neuron*. 2015;88(6):1208-1226.
57. Tilokani L, Russell FM, Hamilton S, et al. AMPK-dependent phosphorylation of MTFR1L regulates mitochondrial morphology. *Sci Adv*. 2022;8(45):eabo7956.
58. Zhang S, Yu Q, Li Z, Zhao Y, Sun Y. Protein neddylation and its role in health and diseases. *Signal Transduct Target Ther*. 2024;9(1):85.
59. Shepherd CE, Goyette J, Utter V, et al. Inflammatory S100A9 and S100A12 proteins in Alzheimer's disease. *Neurobiol Aging*. 2006;27(11):1554-1563.
60. Roque C, Mendes-Oliveira J, Duarte-Chendo C, Baltazar G. The Role of G protein-coupled estrogen receptor 1 on neurological disorders. *Front Neuroendocrinol*. 2019;55:100786.
61. Prossnitz ER, Barton M. The G protein-coupled oestrogen receptor GPER in health and disease: an update. *Nat Rev Endocrinol*. 2023;19(7):407-424.
62. Wang C, Uray IP, Mazumdar A, Mayer JA, Brown PH. SLC22A5/OCTN2 expression in breast cancer is induced by estrogen via a novel intronic estrogen-response element (ERE). *Breast Cancer Res Treat*. 2012;134(1):101-115.
63. Munshi MI, Yao SJ, Ben Mamoun C. Redesigning therapies for pantothenate kinase-associated neurodegeneration. *J Biol Chem*. 2022;298(3):101577.
64. Baltanás FC, Zarich N, Rojas-Cabañeros JM, Santos E. SOS GEFs in health and disease. *Biochim Biophys Acta Rev Cancer*. 2020;1874(2):188445.
65. van Exel E, et al. Cognitive function in the oldest old: women perform better than men. *J Neurol Neurosurg Psychiatry*. 2001;71(1):29-32.
66. Golchert J, Roehr S, Luck T, et al. Women outperform men in verbal episodic memory even in oldest-old age: 13-year longitudinal results of the AgeCoDe/AgeQualiDe Study. *J Alzheimers Dis*. 2019;69(3):857-869.
67. Jack CR, Wiste HJ, Weigand SD, et al. Age, Sex, and APOE epsilon4 effects on memory, brain structure, and beta-amyloid across the adult life span. *JAMA Neurol*. 2015;72(5):511-519.
68. Sohn D, Shpanskaya K, Lucas JE, et al. Sex differences in cognitive decline in subjects with high likelihood of mild cognitive impairment due to Alzheimer's disease. *Sci Rep*. 2018;8(1):7490.
69. Laws KR, Irvine K, Gale TM. Sex differences in cognitive impairment in Alzheimer's disease. *World J Psychiatry*. 2016;6(1):54-65.
70. Oveisgharan S, Arvanitakis Z, Yu L, Farfel J, Schneider JA, Bennett DA. Sex differences in Alzheimer's disease and common neuropathologies of aging. *Acta Neuropathol*. 2018;136(6):887-900.
71. Liesinger AM, Graff-Radford NR, Duara R, et al. Sex and age interact to determine clinicopathologic differences in Alzheimer's Disease. *Acta Neuropathol*. 2018;136(6):873-885.
72. Lim K-H, Yang S, Kim S-H, Ko E, Kang M, Joo J-Y. Cryptic mutations of PLC family members in brain disorders: recent discoveries and a deep-learning-based Approach. *Brain*. 2023;146(4):1267-1280.
73. Su R, Han Z-Y, Fan Ji-P, Zhang Y-L. A possible role of myristoylated alanine-rich c kinase substrate in endocytic pathway of Alzheimer's disease. *Neurosci Bull*. 2010;26(4):338-344.
74. Jurkiewicz D, Michalec K, Skowronek K, Nałęcz KA. Tight junction protein ZO-1 controls organic cation/carnitine transporter OCTN2 (SLC22A5) in a protein kinase C-dependent Way. *Biochim Biophys Acta Mol Cell Res*. 2017;1864(5):797-805.
75. Morshed N, Lee MJ, Rodriguez FH, Lauffenburger DA, Mastroeni D, White FM. Quantitative phosphoproteomics uncovers dysregulated kinase networks in Alzheimer's Disease. *Nat Aging*. 2021;1(6):550-565.
76. Tagawa K, Homma H, Saito A, et al. Comprehensive phosphoproteome analysis unravels the core signaling network that initiates the earliest synapse pathology in preclinical Alzheimer's disease brain. *Hum Mol Genet*. 2015;24(2):540-558.
77. Cristóvão JS, Gomes CM. S100 proteins in Alzheimer's disease. *Front Neurosci*. 2019;13:463.
78. Sim K-Y, Park S-H, Choi KY, et al. High-throughput epitope profiling of antibodies in the plasma of Alzheimer's disease patients using random peptide microarrays. *Sci Rep*. 2019;9(1):4587.
79. Zhao L, Mao Z, Woody SK, Brinton RD. Sex differences in metabolic aging of the brain: insights into female susceptibility to Alzheimer's Disease. *Neurobiol Aging*. 2016;42:69-79.
80. Higginbotham L, Ping L, Dammer EB, et al. Integrated proteomics reveals brain-based cerebrospinal fluid biomarkers in asymptomatic and symptomatic Alzheimer's disease. *Sci Adv*. 2020;6(43).
81. Johnson ECB, Dammer EB, Duong DM, et al. Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. *Nat Med*. 2020;26(5):769-780.
82. Haque R, Watson CM, Liu J, et al. A protein panel in cerebrospinal fluid for diagnostic and predictive assessment of Alzheimer's Disease. *Sci Transl Med*. 2023;15(712):eadg4122.
83. Seifar F, Fox EJ, Shantaraman A, et al. Large-scale deep proteomic analysis in Alzheimer's disease brain regions across race and ethnicity. *Alzheimers Dement*. 2024;20(12):8878-8897.
84. Kruse AC, Kobilka BK, Gautam D, Sexton PM, Christopoulos A, Wess J. Muscarinic acetylcholine receptors: novel opportunities for drug development. *Nat Rev Drug Discov*. 2014;13(7):549-560.
85. Patsopoulos NA, Tatsioni A, Ioannidis JP. Claims of sex differences: an empirical assessment in genetic associations. *JAMA*. 2007;298(8):880-893.
86. Dumitrescu L, Barnes LL, Thambisetty M, et al. Sex differences in the genetic predictors of Alzheimer's pathology. *Brain*. 2019;142(9):2581-2589.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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