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RESEARCH ARTICLE

Distinct inflammatory profiles in young-onset versus late-onset Alzheimer's disease

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Abstract

INTRODUCTION: Neuroinflammation, a key player in Alzheimer's disease (AD) pathogenesis, may be differentially involved in young-onset (YOAD) compared to late-onset (LOAD) AD.

METHODS: Using proximity extension assay technology, we examined 737 inflammatory markers in the CSF of 26 healthy controls (63.9 ± 8.7; 12♀), 57 patients with YOAD (60.8 ± 4.9 y/o; 40♀), and 33 with LOAD (76.6 ± 4.5 y/o; 18♀). We also assessed biomarkers of AD pathology (Aβ42, p-tau181, t-tau) and neurodegeneration (neurofilament light-chain [NfL]).

RESULTS: Compared to controls, SCRN1 and MMP10 were increased in LOAD and YOAD, but 16 markers showed YOAD-specific increases. Forty-six markers were significantly associated with NfL. P-tau181 and t-tau mediated the association between

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inflammatory markers and NfL in YOAD. In LOAD we could not identify a direct or indirect relationship between neuroinflammation and neurodegeneration.

DISCUSSION: Using a proteomics approach, we observed an exacerbation of neuroinflammatory changes and a differential contribution of neuroinflammation to AD pathology and neurodegeneration in YOAD compared to LOAD.

KEYWORDS

biofluids, biomarkers, cerebrospinal fluid, early-onset Alzheimer's disease, late-onset Alzheimer's disease, neuroinflammation, proteomics, young-onset Alzheimer's disease

Highlights

- Olink's Proximity Extension Assay was used to compare the inflammatory profile of 26 healthy controls and 90 Alzheimer's disease (AD) patients.
- AD patients were further stratified into young-onset (YOAD, $n = 57$) and late-onset (LOAD, $n = 33$) AD.
- Cerebrospinal fluid (CSF) levels of MMP10 and SCRN1 were increased in both YOAD and LOAD, but 16 proteins were only increased in YOAD.
- Tau mediated the association between inflammatory markers and neurodegeneration in YOAD.
- Neuroinflammation may be differentially involved in the pathogenesis of YOAD compared to LOAD.

1 | BACKGROUND

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive cognitive impairment and neuropathological hallmarks, that is, the deposition of amyloid β (Aβ) plaques and tau-containing neurofibrillary tangles (NFTs).[1] Age is the main risk factor for AD, but in 5%–10% of cases symptoms develop by age 65, which is known as early- or young-onset AD (YOAD), in contrast with late-onset AD (LOAD).[2,3] Although Aβ plaques and NFTs are present in both YOAD and LOAD, YOAD patients may show a more aggressive disease course with a relatively short survival time,[2] as well as more severe plaque and neurofibrillary tangle (NFT) burden,[4–8] gray matter loss,[9] cortical atrophy,[10] synaptic loss,[6,11] and perfusion and metabolic deficits.[12] These differences would be consistent with an exacerbation of AD-related pathophysiological mechanisms in YOAD. Neuroinflammation, in particular, is now recognized as a key player in AD pathophysiology,[13] but little is known about how age mediates its relationship with neurodegeneration.

Numerous pathological, positron emission tomography (PET), and biofluid studies have reported microglial activation and astrogliosis in AD.[13–17] Once thought to be a bystander effect of Aβ plaques and NFTs, these neuroinflammatory processes likely contribute to as well as exacerbate Aβ- and tau-mediated neurodegeneration. Microglia, the resident phagocytes of the central nervous system, switch to an activated state to remove pathological triggers like plaques through phagocytosis and proinflammatory cytokine release.[13,18] However, sustained neuroinflammation and cytokine release can impair their phagocytic function and lead to neurotoxicity.[13,18] Microglial activation has thus been associated with focal gray matter atrophy, Aβ load, PET tau burden, and cognitive decline, prompting trials of immunomodulatory strategies in AD.[18–21] Similarly, Aβ plaques and tau pathology disrupt the neuroprotective functions of astrocytes, which depend on well-regulated cytokine secretion.[13] Proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin (IL) -1β, IL-6, and IL-18, are elevated in the brain and blood of AD patients.[22–29]

A limited number of pathological studies has characterized neuroinflammation in YOAD patients. In a post-mortem analysis of AD brains at similar Braak stages, Yin et al. 2017 revealed an increase in Aβ plaque-associated hyperreactive microglia, with higher expression of phagocytic genes, in YOAD compared to LOAD.[30] Other studies on microglial activation and astrocytic reactivity have reported that the contrast between AD and age-matched control cases decreased with age.[31,32] Unfortunately, biomarker studies, especially in the cerebrospinal fluid (CSF), remain limited in number and scope. Furthermore, only a few inflammatory markers have been investigated, failing to capture the complexity of neuroimmune interactions.[33]

Here we hypothesize that neuroinflammation, a central player in AD pathogenesis, could contribute differentially to the pathophysiology of YOAD and LOAD.[13,15,34] We used an exploratory approach to assess the CSF inflammatory profiles of YOAD and LOAD patients, by analyzing 737 inflammatory markers with the highly sensitive proximity extension assay (PEA) technology.[35] We also examined the correlation between inflammation and neurodegeneration, in both YOAD and

LOAD patients. We hypothesized that while immune dysregulation may be a shared feature in YOAD and LOAD, a more distinct and severe pattern may be seen in YOAD.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional (PubMed, OVID Medline) sources. Neuroinflammation is a key player in Alzheimer's disease (AD) pathogenesis, but whether it is differentially involved in the pathogenesis of young-onset (YOAD) compared to late-onset (LOAD) AD remains a critical gap in knowledge. We used a proteomics-based approach to characterize the inflammatory profile of YOAD versus LOAD patients.
2. **Interpretation**: Although increases in cerebrospinal fluid (CSF) levels of MMP10 and SCRN1 were shared in YOAD and LOAD compared to healthy controls, we report YOAD-specific increases for 16 other inflammatory markers. The relationship between inflammation and neurodegeneration was mediated by tau pathology in YOAD but not LOAD. Our findings suggest an exacerbation of neuroinflammatory changes in YOAD compared to LOAD, which may impact response to disease-modifying treatments.
3. **Future directions**: Further investigation is needed to characterize the biological pathways implicated in these differences, and the effect of known mediators of neuroinflammation, such as sex.

2 | METHODS

2.1 | Participants

All patients presenting with cognitive impairment who underwent a lumbar puncture for research purposes at the University Health Network (UHN) Memory Clinic (Toronto, Ontario, CA) between 2013–2023 were considered for inclusion. Final inclusion was based on AD biomarker positivity (Aβ-PET-or CSF) and a clinical diagnosis of typical or atypical AD. Clinical diagnoses included AD dementia, mild cognitive impairment (MCI), logopenic variant (lvPPA), and nonfluent variant (nfvPPA) primary progressive aphasia, frontal variant AD (fvAD), posterior cortical atrophy (PCA), and corticobasal syndrome (CBS) (See Table 1). We subdivided our AD cohort based on their age at onset into YOAD (age at onset $\leq$ 65 years) and LOAD. The healthy control (HC) cohort included 6 cognitively normal individuals recruited at UHN as part of a research study on frontotemporal lobar degeneration and twenty others recruited as part of the National Institutes of Health (NIH) grant P30AG062421 at the Massachusetts General Hospital (MGH) and Harvard Medical School, Boston (Massachusetts, USA). All HC were CSF AD-biomarker negative. Apolipoprotein E (APOE) status was determined by mass spectrometry for a subset of 20 HC,[36] and by genotyping for a subset of 3 HC, 15 LOAD, and 47 YOAD participants.[37] These participants were classified into APOE4-positive ($\geq$1 APOE ε4 allele) and APOE4-negative status.

2.2 | CSF collection

CSF collection and pre-analytical processing was performed as previously described.[38] Samples underwent one more freeze-thaw cycle prior to the proteomic assay (inflammatory markers).

2.3 | Cognitive scores

Cognitive assessments of AD patients included the Montreal Cognitive Assessment (MoCA) ($N = 65$), Alzheimer's Disease Comprehensive Scoring-Cognition (ADAS-Cog) ($N = 16$), Toronto Cognitive Assessment (TorCA) ($N = 4$), and Mini-Mental State Examination (MMSE) ($N = 1$), performed within 6 months of the LP. All results were z-scored using their respective normative population references.[39–42]

2.4 | Inflammatory markers

The levels of 737 inflammatory markers (Olink Explore Inflammation panels I and II) were measured using PEA technology (Olink Proteomics, Boston, USA) at Hamilton Health Sciences (Hamilton, ON, Canada).[35] The read-out was obtained as a Normalized Protein eXpression (NPX) value on a log2-scale. To combine data from two separate runs, subset normalization was applied to the NPX of each protein, using the difference of medians of the HC present on each run (https://cran.r-project.org/web/packages/OlinkAnalyze/vignettes/Vignett.html).

2.5 | AD biomarkers

For participants recruited at UHN, sandwich enzyme-linked immunosorbent assays (ELISAs) were used to measure concentrations of Aβ42 (Innotest β-amyloid (1-42), Fujirebio, USA), phosphorylated tau (p-tau) 181 (p-tau181) (Innotest phospho-tau (181p), Fujirebio), and t-tau (Innotest hTAU-Ag, Fujirebio). AD biomarker positivity was defined as: p-tau181 > 68 pg/mL and Aβ42 to tau Index (ATI) < 0.8 (ATI being defined as: Aβ42/(240+1.18*t-tau).[38] A cutoff of 20% was used for the maximum inter- and intra-assay coefficient of variation (CV) of each run. Mean inter-assay CVs were respectively 9.8 ± 5.5%, 8.0 ± 4.3%, and 10.1 ± 5.9% and mean intra-assay CVs were 1.9 ± .3%, 2.4 ± .6%, and 4.1 ± 1.2% for the p-tau181, t-tau, and Aβ42 respectively. For participants recruited at the MGH and Harvard Medical School, Aβ40 (Beta-Amyloid (1-40) Assay), Aβ42 (Beta-Amyloid (1-42)

TABLE 1 Demographics, clinical characteristics, and CSF biomarkers of the cohort used in this study

Parameter	YOAD N = 57	LOAD N = 33	HC N = 26	*p*-value
Clinical subtypes	25 typical AD dementia (43.9%); 12 aMCI (21.1%); 9 CBS (15.8%); 7 PPA (lvPPA, nfvPPA, or MCI-language) (12.3%); 2 fvAD (MCI/dementia) (3.5%); and 2 PCA (MCI/dementia) (3.5%)	20 typical AD dementia (60.6%); 4 aMCI (12.1%); 9 PPA (lvPPA, nfvPPA, or MCI-language) (27.3%);	N/A	ns[b]
Age (years) (mean ± S.D)	60.8 ± 4.9[#]	76.6 ± 4.5	63.9 ± 8.7	<0.0001[a]
Sex (female/male)	40/17	18/15	12/14	ns (0.084)[b]
Disease duration (years) (mean ± S.D)	4.0 ± 2.7	3.4 ± 2.8	N/A	ns[c]
Cognitive score (z-scored) (mean ± S.D)	−8.5 ± 4.3 (n = 55)	−5.7 ± 3.6 (n = 31)	N/A	<0.01[c]
CSF biomarkers				
Aβ42 (pg/mL) (mean ± S.D)	361.9 ± 130.4	456.21 ± 140.86 (n = 30)		<0.01[c]
P-tau181 (pg/mL) (mean ± S.D)	108.9 ± 33.5	110.0 ± 27.3 (n = 30)	33.3 ± 11.4	ns[c]
Total tau (pg/mL) (mean ± S.D)	877.7 ± 385.3	825.3 ± 377.3 (n = 30)	192.6 ± 51.6	ns[c]
NfL (pg/mL) (mean ± S.D)	1,641.3 ± 541.5[*]	1,966.9 ± 977.0	686.4 ± 278.9	<0.0001[d]

[a] Kruskal-Wallis test.

[b] Chi-squared test of independence without continuity correction.

[c] Wilcoxon Rank Sum test.

[d] ANCOVA with age as covariate.

[#] No difference between YOAD and HC.

[*] No difference between YOAD and LOAD.

Note: The chi-squared test on clinical subtypes was performed on the proportion of typical (AD dementia, aMCI) versus atypical (all other syndromes) presentations in YOAD versus LOAD.

Abbreviations: AD, Alzheimer's disease; aMCI, amnestic mild cognitive impairment; Aβ42, β-amyloid 1-42; CBS, corticobasal syndrome; LOAD, late-onset Alzheimer's disease; lvPPA, logopenic variant primary progressive aphasia; MCI, mild cognitive impairment; NfL, neurofilament light-chain; nfvPPA, nonfluent variant primary progressive aphasia; ns, not significant; PPA, primary progressive aphasia; p-tau181, phosphorylated tau at threonine 181; SD, standard deviation; YOAD, young-onset Alzheimer's disease.

Assay), p-tau181 (P-Tau (pT181) Assay), and t-tau (Total Tau Assay) levels were measured using ELISA from Euroimmun, Germany.

2.6 | Neurofilament light-chain

Neurofilament light-chain (NfL) levels were measured using a commercially available NF-light SIMOA Assay Advantage kit or the Neurology 2-Plex B assay kit (Quanterix, USA) on a single molecule array (Simoa) SR-X analyzer according to manufacturer's instructions.

2.7 | Statistical analysis

All data processing and analyses were conducted in R (4.2.2). We ran analyses of covariance (ANCOVAs) with diagnosis (YOAD, LOAD or HC) as independent variables, NPX as the outcome variable, and age and sex as covariates.

Pathway enrichment analysis was done using the integrated pathway database pathDIP (https://ophid.utoronto.ca/pathDIP) version 5.0.32.4 (Database version 5.0.31.0).[43] All proteins showing significant differences between groups prior to the multiple comparison correction were used as input for the pathway analysis. An enrichment score (ES) ratio was calculated by dividing the number of proteins annotated with a pathway by the total number of proteins annotated with any pathway.

To investigate the effect of AD subtype (YOAD versus LOAD) on the relationship between NPX and NfL (or cognitive scores) we ran multivariable linear regressions incorporating an interaction of AD subtype with NPX (or cognitive scores) on NfL. We also performed mediation analyses with the package mediation[44] on the subset of proteins that were found to be differentially expressed in AD versus HC. The models, which included age and sex, examined whether the association of the inflammatory markers on NfL was mediated by AD markers. We applied bootstrapping with 1000 iterations to estimate 95% confidence intervals (CIs). Finally, to check whether our findings were confounded by APOE4 status, we reran analyses on the proteins that showed significant differences. In the subset of participants for whom APOE status had been obtained, we compared the results before and after the addition of APOE4 status as a covariate.

False discovery rate (FDR) correction was applied, and the level of statistical significance was set at FDR-adjusted p-value (q-value) < 0.05. Effect sizes were calculated with emmeans (https://CRAN.R-project.org/package=emmeans).

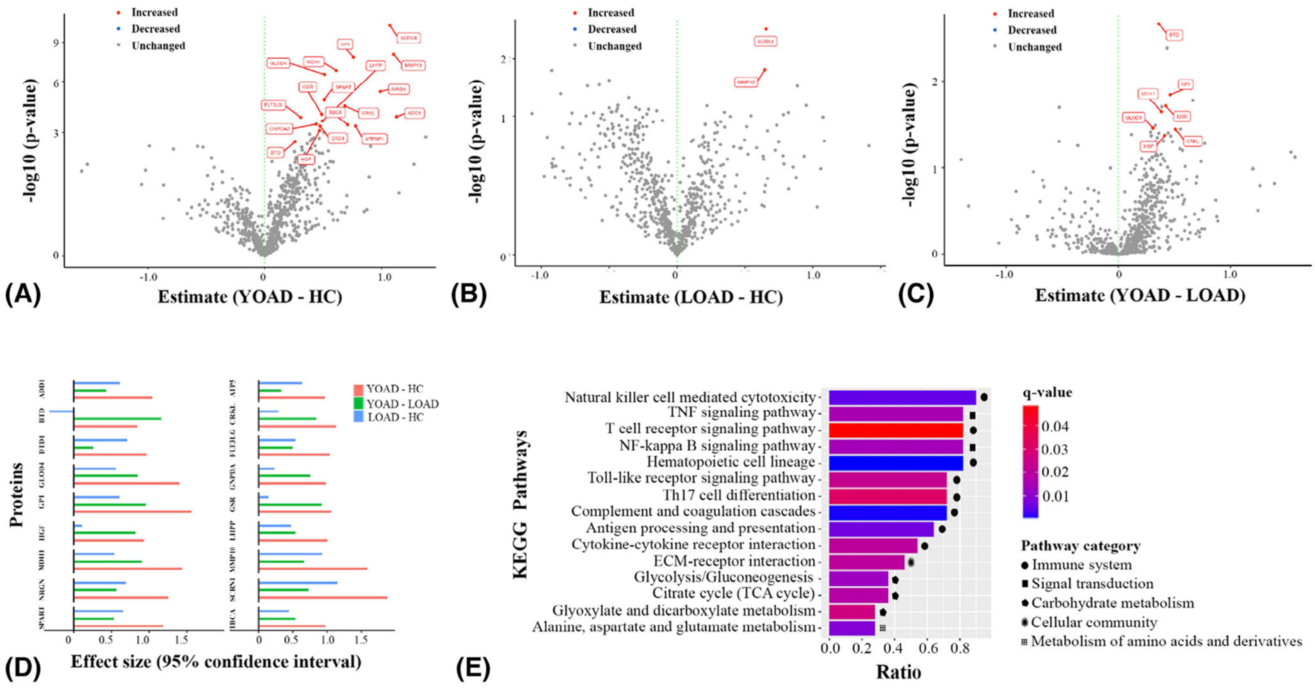

FIGURE 1 Differences in inflammatory profiles between young-onset Alzheimer's disease (YOAD), late-onset AD (LOAD), and healthy controls (HC). (A-C) Volcano plots showing differences in Normalized Protein eXpression (NPX) between (A) YOAD and HC, (B) LOAD and HC, and (C) YOAD and LOAD. Estimated mean differences in NPX are plotted on the x-axis and -log *p*-values on the y-axis. Inflammatory markers significantly different between groups ($q < 0.05$) are labeled. (D) Effect size comparisons for the 18 inflammatory markers that were shown to be differentially expressed in AD compared to HC. The x-axis represents effect size and the y-axis the inflammatory marker. (E) Pathway enrichment analysis shows the pathways that are differentially implicated in YOAD and LOAD. X-axis shows the enrichment scores and the y-axis the pathways.

3 | RESULTS

3.1 | Cohort characteristics

Ninety AD-positive patients (57 YOAD, 33 LOAD) and 26 AD-negative HC were included (Table 1). We found no significant difference in age between YOAD and HC; LOAD patients were older than the HC ($p < 0.001$). There was no significant difference in sex distribution, with female participants representing 70% of YOAD, 55% of LOAD, and 46% of HC ($p > 0.05$).

Compared to LOAD patients, YOAD patients had significantly lower CSF Aβ42 levels ($p < 0.01$), but there were no differences in p-tau181, t-tau, or NfL levels.

3.2 | Inflammatory markers in YOAD, LOAD, and HC

Across the 116 samples, more than 75% data were missing for seven markers (BCL2L11, BID, EP300, FGF3, ADIPOQ, FUOM, MGLL), which were removed from the analysis.

Eighteen inflammatory markers significantly differed across groups ($q < 0.05$), and were further analyzed in post-host tests with a Bonferroni correction for pairwise comparisons. Compared to HC, YOAD showed higher levels of 18 markers: SCRN1 ($d = 1.88$, $p < 0.001$), MMP10 ($d = 1.59$, $p < 0.001$), GPI ($d = 1.60$, $p < 0.001$), MDH1 ($d = 1.44$, $p < 0.001$), GLOD4 ($d = 1.40$, $p < 0.001$), NRGN ($d = 1.25$, $p < 0.001$), SPART ($d = 1.18$, $p < 0.001$), CRKL ($d = 1.13$, $p < 0.001$), GSR ($d = 1.06$, $p < 0.001$), ADD1 ($d = 1.04$, $p < 0.001$), FLT3LG ($d = 1.03$, $p < 0.001$), LHPP ($d = 1.00$, $p < 0.001$), GNPDA2 ($d = 0.98$, $p < 0.001$), TBCA ($d = 0.97$, $p < 0.001$), ATP5IF1 ($d = 0.96$, $p < 0.001$), DTD1 ($d = 0.96$, $p < 0.001$), HGF ($d = 0.93$, $p < 0.001$), and BTD ($d = 0.84$, $p < 0.01$; Figure 1A, 1D, Table 2). LOAD had greater levels of only two markers, SCRN1 ($d = 1.15$, $p < 0.01$) and MMP10 ($d = 0.93$, $p < 0.05$; see Figure 1B and 1D, and Table 2). When comparing YOAD and LOAD, we found differences in the following seven inflammatory markers: BTD ($d = 1.16$, $p < 0.01$), GPI ($d = 0.96$, $p < 0.05$), GSR ($d = 0.92$, $p < 0.05$), MDH1 ($d = 0.90$, $p < 0.05$), GLOD4 ($d = 0.85$, $p < 0.05$), CRKL ($d = 0.84$, $p < 0.05$), and HGF ($d = 0.82$, $p < 0.05$; Figure 1C, 1D, Table 2).

There were diagnosis-specific sex differences in NPX (Table S1). While in the HC 31 markers were upregulated in males compared to females, these markers were not differentially regulated in either YOAD or LOAD. Instead, in YOAD it was females who showed increased levels of 13/15 other proteins, including: GZMA, APCS, SIT1, PGF, EIF4E. In LOAD, only four proteins were differentially regulated between males and females: ANGPTL2

TABLE 2 Differentially expressed CSF proteins among YOAD, LOAD, and HC

Protein	Protein name	YOAD versus HC			LOAD versus HC			YOAD versus LOAD		
		Estimate (beta)	Effect size (95% CI)	*p*-value	Estimate (beta)	Effect size (95% CI)	*p*-value	Estimate (beta)	Effect size (95% CI)	*p*-value
SCRN1	Secernin-1	1.07	1.88 (1.39–2.37)	3.14E-11	0.65	1.15 (0.49–1.81)	0.0023	0.42	0.73 (0.06–1.40)	0.1001
MMP10	Matrix metallo-proteinase-10	1.10	1.59 (1.10–2.08)	9.37E-09	0.64	0.93 (0.27–1.58)	0.0181	0.46	0.66 (-0.005–1.33)	0.1546
GPI	Glucose-6-phosphate isomerase	0.76	1.56 (1.07–2.05)	1.6E-08	0.29	0.60 (-0.05–1.26)	0.2123	0.47	0.96 (0.29–1.62)	0.0157
MDH1	Malate dehydrogenase	0.61	1.44 (0.95–1.92)	1.68E-07	0.23	0.54 (-0.12–1.19)	0.3272	0.38	0.90 (0.24–1.57)	0.0250
GLOD4	Glyoxalase domain-containing protein 4	0.51	1.40 (0.91–1.89)	3.25E-07	0.20	0.55 (-0.10–1.21)	0.2905	0.31	0.85 (0.18–1.51)	0.0393
NRGN	Neurogranin	0.99	1.25 (0.76–1.74)	4.64E-06	0.54	0.69 (0.03–1.35)	0.1193	0.44	0.56 (-0.10–1.23)	0.2894
SPART	Spartin	0.51	1.18 (0.70–1.67)	1.48E-05	0.28	0.65 (0.00–1.31)	0.1533	0.23	0.53 (-0.14–1.20)	0.3506
CRKL	Crk-like protein	0.69	1.13 (0.64–1.62)	3.73E-05	0.17	0.29 (-0.37–0.94)	1	0.51	0.84 (0.18–1.51)	0.0407
GSR	Glutathione reductase	0.49	1.06 (0.57–1.55)	0.0001	0.06	0.14 (-0.52–0.8)	1	0.42	0.92 (0.26–1.58)	0.0213
ADD1	Alpha-adducin	1.13	1.04 (0.55–1.53)	0.00015	0.66	0.61 (-0.05–1.27)	0.2022	0.46	0.43 (-0.24–1.09)	0.6171
FLT3LG	Fms-related tyrosine kinase 3 ligand	0.31	1.03 (0.55–1.52)	0.00017	0.16	0.54 (-0.12–1.19)	0.3262	0.15	0.50 (-0.17–1.16)	0.4221
LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	0.49	1.00 (0.52–1.49)	0.00026	0.23	0.47 (-0.19–1.13)	0.4786	0.26	0.54 (-0.13–1.20)	0.3413
GNPDA2	Glucosamine-6-phosphate isomerase 2	0.44	0.98 (0.49–1.47)	0.00039	0.10	0.23 (-0.43–0.88)	1	0.34	0.75 (0.09–1.42)	0.0813
TBCA	Tubulin-specific chaperone A	0.71	0.97 (0.48–1.46)	0.00042	0.32	0.44 (-0.22–1.1)	0.5647	0.40	0.53 (-0.13–1.20)	0.3421
ATP5IF1	ATPase inhibitor	0.78	0.96 (0.47–1.45)	0.00049	0.51	0.63 (-0.02–1.29)	0.1754	0.27	0.33 (-0.34–0.99)	0.9879
DTD1	D-aminoacyl-tRNA deacylase 1	0.47	0.96 (0.47–1.45)	0.00049	0.35	0.71 (-0.05–1.36)	0.1053	0.13	0.25 (-0.41–0.92)	1
HGF	Hepatocyte growth factor	0.47	0.93 (0.44–1.42)	0.00082	0.05	0.11 (0.55–0.76)	1	0.42	0.82 (0.16–1.48)	0.0484
BTD	Biotinidase	0.26	0.84 (0.35–1.33)	0.00279	−0.10	−0.32 (-0.98–0.33)	0.9946	0.36	1.16 (0.50–1.83)	0.0023

Note: *p*-value corresponds to the Bonferroni corrected *p*-value during post-hoc test to compare between the groups (YOAD, LOAD, and HC) for only the proteins which were significantly different among the groups after FDR 5% correction.

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; HC, healthy control; LOAD, late-onset Alzheimer's disease; YOAD, young-onset Alzheimer's disease.

and SCG3 (higher in males), and IL2RB and DAPK2 (higher in females).

3.3 | Pathway enrichment analysis

A comprehensive pathway enrichment analysis across all source databases showed that the most important differences between YOAD and LOAD were for the immune system category ($n = 110$), with carbohydrate metabolism as distant second ($n = 36$). Query of the KEGG database revealed that pathways differentially affected in YOAD and LOAD primarily involved immune and cellular responses, metabolism, and signal transduction, including: the complement and coagulation cascades (q-value < 0.001; ES $= 0.36$), cytokine-cytokine receptor interaction (q-value $= 0.021$; ES $= 0.27$), extracellular matrix-receptor interactions (q-value $= 0.020$; ES $= 0.23$), glycolysis/gluconeogenesis (q-value $= 0.013$; ES $= 0.18$), and tricarboxylic acid cycle (q-value $= 0.018$; ES $= 0.18$) (Figure 1E).

3.4 | Relationship between inflammatory markers and markers of neurodegeneration

Multivariable linear regressions adjusting for age and sex showed that 46 inflammatory markers were associated with NfL in AD patients ($q < 0.05$; Table S2). Among them, SPART, GLOD4, and MDH1 were increased in AD compared to HC.

We then ran multivariable linear regressions with an interaction term of AD subtype and NPX as the independent variable, and the following dependent variables (in separate models, all adjusted for age and sex): NfL, Aβ42, p-tau181 or t-tau (all log-transformed). There was a significant interaction of GIT1 ($\beta \pm$ SE: $0.33 \pm .08$, $q < 0.05$) and KLRD1 ($\beta \pm$ SE:$.33 \pm .08$, $q < 0.05$) with AD subtype on NfL levels. Slope visualization suggested a stronger positive relationship of GIT1 and KLDR1 with NfL in LOAD compared to YOAD. There was no significant interaction of any inflammatory marker with AD subtype on AD markers.

3.5 | Relationship between inflammatory markers and cognition in YOAD and LOAD

Wilcoxon-rank sum found that LOAD patients had higher z-scores than YOAD patients (-5.7 ± 3.6 and -8.5 ± 4.3, respectively), indicating better performance relative to age- and sex-matched HC ($p < 0.05$) (Table 1). Linear regressions showed that these differences were attributable to age ($\beta \pm$ SE: $0.37 \pm .09$, $p < 0.001$) and not to other variables such as Aβ42 ($\beta \pm$ SE: $0.00 \pm .00$, $p > 0.05$), p-tau181 ($\beta \pm$ SE: $0.01 \pm .01$, $p > 0.05$), t-tau ($\beta \pm$ SE: $-0.00 \pm .00$, $p > 0.05$), or NfL ($\beta \pm$ SE: $0.00 \pm .00$, $p > 0.05$). We also found no interaction effect of any inflammatory marker with AD subtype on cognitive z-scores.

3.6 | Mediation of the relationship between inflammation and neurodegeneration by AD biomarkers

We examined whether AD biomarkers in YOAD and LOAD mediated the association of the 18 differentially expressed inflammatory markers on neurodegeneration. This was carried out through mediation analyses adjusted for age and sex, with NPX as the independent variable, AD biomarkers as the mediator, and logged NfL as the dependent variable.

In YOAD, a positive total association (direct effect plus mediation effect of p-tau181) was found for eight markers and was mediated by p-tau181 (Figure 2). The indirect effects of SCRN1 (mediation effect: 0.09; $q < 0.05$), NRGN (0.17; $q < 0.001$), MMP10 (0.06; $q < 0.05$), SPART (0.11; $q < 0.05$), GNPDA2 (0.10; $q < 0.05$), MDH1 (0.17; $q < 0.05$), LHPP (0.11; $q < 0.05$), and ATP5IF1 (0.11; $q < 0.05$) on NfL via CSF p-tau181 were significant. In LOAD, these markers showed no direct or indirect relationship with NfL.

We observed a similar pattern for CSF t-tau, which mediated the effect of eight markers on NfL in YOAD but not LOAD: SCRN1 (mediation effect: 0.10; $q < 0.05$), MDH1 (0.15; $q < 0.001$), NGRN (0.14; $q < 0.001$), SPART (0.10; $q < 0.05$), LHPP (0.11; $q < 0.001$), GNPDA2 (0.12; $q < 0.01$), ATP5IF1 (0.10; $q < 0.05$), and HGF (0.08; $q < 0.05$) (Figure 3).

Aβ42 was not a mediator of the association between differentially expressed markers and NfL in either YOAD or LOAD.

3.7 | Effect of APOE4

In the subset of individuals with APOE status, which included 23 HC (5 APOE4-positive), 15 LOAD (8 APOE4-positive), and 47 YOAD (21 APOE4-positive) participants, we found no association between AD subtype and APOE4 positivity per the chi-squared test ($p > 0.05$). We then investigated whether the 18 differentially regulated proteins still showed differences between HC, YOAD, and LOAD, after controlling for APOE4 status.

Likely due to sample size, we could not reproduce all the findings in the subset of participants with APOE status. We found no significant difference in YOAD versus HC for ATP5IF, and of LOAD versus HC for MMP10. The other significant differences reported in the full dataset could be reproduced in this subset. This remained the case after adding APOE4 status as a covariate in the model ($q < 0.05$). Similarly, the mediation effects reported in Figure 2 and Fig 3 remained significant after adding APOE4 status as a covariate ($q < 0.05$).

4 | DISCUSSION

In this study, we used the Olink PEA technology[35] to assess neuroinflammation in the CSF of 57 YOAD, 33 LOAD, and 26 HC participants. We found that 2 inflammatory markers were increased in both YOAD

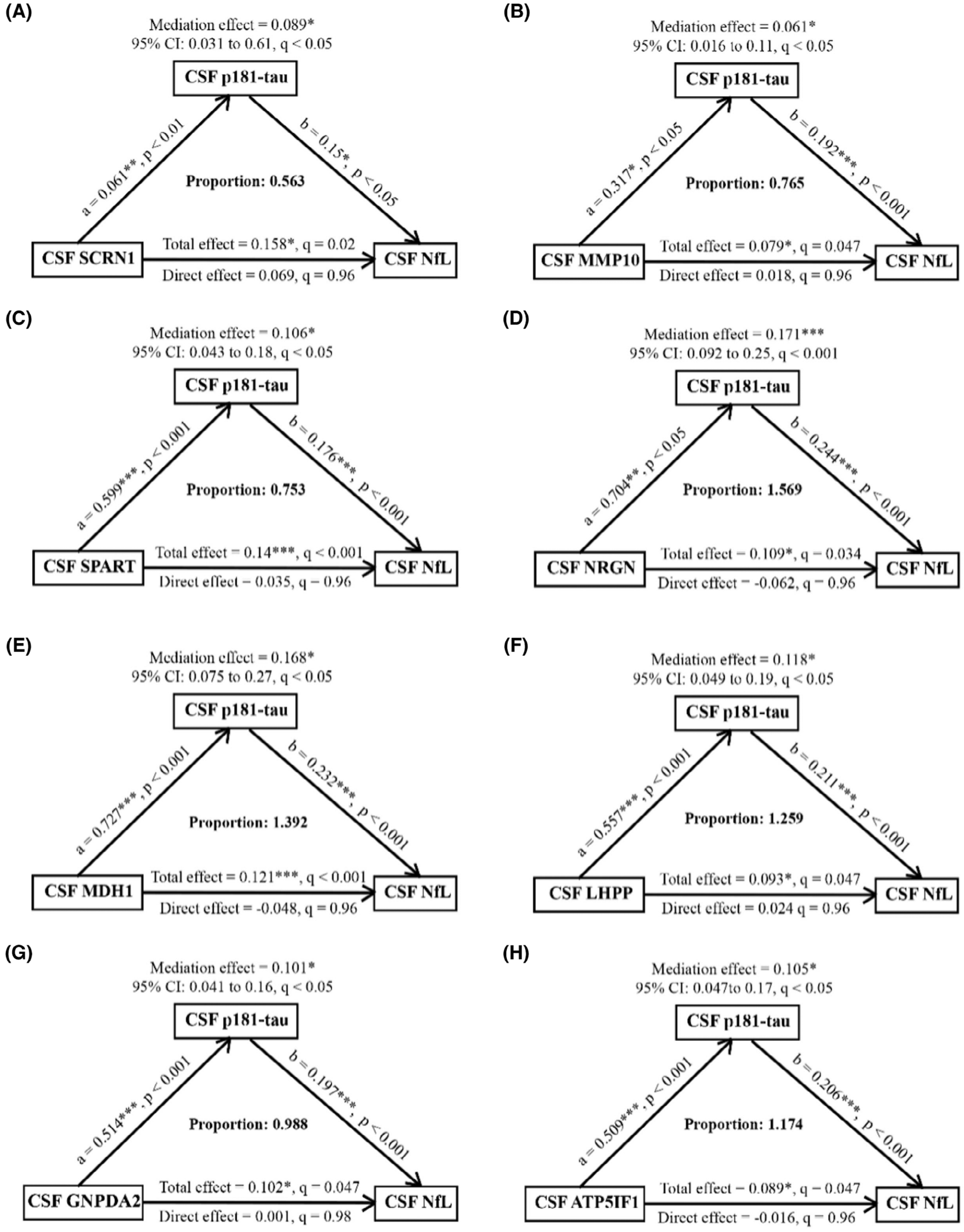

FIGURE 2 The association of the differentially regulated inflammatory markers with neurofilament light-chain (NfL) is mediated by phosphorylated tau 181 (p-tau181). In young-onset Alzheimer's disease (YOAD), p-tau181 significantly mediates the association of (A) SCRN1, (B) MMP10, (C) SPART, (D) NGRN, (E) MDH1, (F) LHPP, (G) GNPDA2, and (H) ATP5IF1 with NfL levels. In each plot, "a" is the effect of the inflammatory marker on the mediator, p-tau181; "b" is the effect of mediator p-tau181 on NfL; "Total effect" indicates the total effect that a given inflammatory marker has on NfL, which is the sum of the "Direct effect" with the "Mediation effect;" "Proportion" is the proportion of the association of the inflammatory marker with NfL that is mediated by p-tau181.

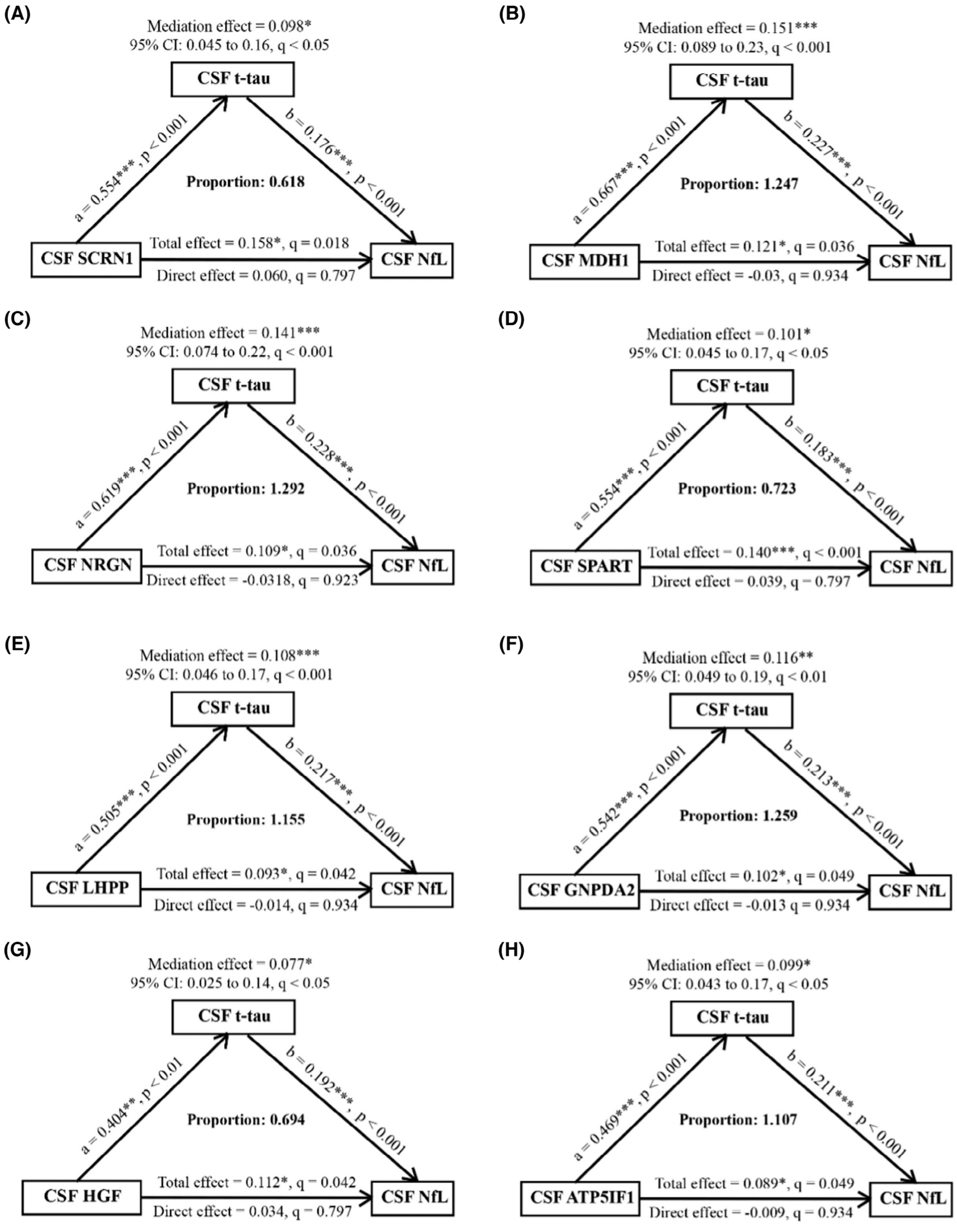

FIGURE 3 The association of the differentially regulated inflammatory marker with neurofilament light-chain (NfL) is mediated by total tau (t-tau). T-tau significantly mediates the association of (A) SCRN1, (B) MDH1, (C) NRGN, (D) SPART, (E) LHPP, (F) GNPDA2, (G) HGF, and (H) ATP5IF1 with NfL.

and LOAD compared to HC, 16 others in YOAD only compared to HC, and 7 in YOAD compared to LOAD. Further probing of the 18 differentially expressed markers in AD versus HC, showed that p-tau181 and t-tau levels mediated the relationship between some of these inflammatory markers and NfL, but only in YOAD. In LOAD, no relationship, direct or indirect, was found between neuroinflammation and neurodegeneration. These results support our hypothesis that YOAD and LOAD have distinct inflammatory profiles, with different contributions from inflammatory processes to neurodegeneration. To our knowledge, this is the first time that a large number of inflammatory markers are compared in the CSF of YOAD and LOAD.

Elevated levels of the matrix metalloproteinase MMP10 and of SCRN1 were detected in both YOAD and LOAD. Matrix metalloproteinases, expressed in neurons and secreted by microglia and astrocytes, contribute to protein degradation in the extracellular matrix. Increased CSF MMP10 levels in particular are thought to reflect disruption of the blood-brain-barrier,[45,46] have been reported across AD stages, and have been associated with faster clinical progression.[45–48] SCRN1, also elevated in both LOAD and YOAD, is an AD-specific p-tau binding protein, with prior studies linking it to NFT pathology in AD but not in other tauopathies.[49,50] In this cohort, SCRN1 showed no association with CSF p-tau181, suggesting it interacts specifically with insoluble aggregated tau in brain tissue, rather than soluble p-tau181.

Sixteen inflammatory markers were significantly increased in YOAD, but not LOAD, compared to HC: GPI, MDH1, NRGN, SPART, CRKL, GSR, GLOD4, ADD1, FLT3LG, LHPP, GNPDA2, TBCA, ATP5IF1, DTD1, HGF, and BTD. Of these, seven (GPI, MDH1, CRKL, GSR, GLOD4, HGF, and BTD) were significantly higher in YOAD than LOAD. Previous studies had linked NRGN, SPART, CRKL, GSR, GLOD4, TBCA, MDH1, and ATP5IF1 to MCI due to AD but did not report any age-related differences.[51] Some of these YOAD-specific increases may reflect an age-dependent compensatory mechanism against neurodegeneration. For instance, while others have reported BTD downregulation in AD plasma,[52,53] here we detected elevated BTD CSF levels in YOAD compared to HC. BTD is involved in biotin metabolism and BTD deficiency leads to demyelination and axonal degeneration.[54,55] GLOD4 is also neuroprotective and involved in cell cycle control.[54] Increased levels of MDH1, which is critical for mitochondrial respiration,[56,57] could reflect a more prominent role for energy dysfunction in YOAD pathophysiology. Lower protein levels in LOAD may also result from declining protein turnover with age,[58] as biomarker levels for neuronal injury often plateau or even decrease in advanced LOAD.[59] Finally, glial response and response to pathological injury are also known to deteriorate with age.[13] Hoozemans et al. (2011) thus proposed that differences in neuroinflammatory markers between AD and controls diminish with age and that there is a more robust correlation between neuroinflammation and AD pathology in young AD patients compared to older ones.[32] In keeping with this, we found elevated HGF levels in YOAD. HGF is increased in astrocytes and microglia associated with senile plaques, contributes to microglial response to central nervous system (CNS) injuries, and has been associated with magnetic resonance imaging (MRI) white matter hyperintensities in AD.[45,60–62] Our pathway enrichment analysis also highlighted cytokine and chemokine enrichment in YOAD compared to LOAD.

This study also aimed to characterize the relationship between neuroinflammation and AD-related neurodegeneration. In our AD cohort, 47 inflammatory markers were associated with NfL, a marker of neuroaxonal damage, but none with Aβ42, p-tau181, or t-tau. However, p-tau181 and t-tau partially mediated the relationship between inflammatory markers with NfL in YOAD and not in LOAD. Consistent with these findings, a PET study showed a stronger correlation of neuroinflammation with tau than Aβ in MCI.[63]

Several studies point toward a sexual dimorphism in the neuroinflammatory processes implicated in AD, likely exacerbated in females compared to males.[64,65] Here, we found that the sexual dimorphism observed in HC, whereby males showed higher levels of 31 markers than females, was disrupted in the two AD groups. In fact, in YOAD, females showed a pattern of upregulation compared to males. Most of these differently regulated proteins had previously been implicated in AD pathophysiology.[66–68] Of note, while we controlled for sex in our analyses, sex differences in neuroinflammatory processes may still confound the increase in inflammatory markers we report in YOAD compared to LOAD—since the former had a higher (albeit not significant) proportion of females.

Other study limitations include a small sample size relative to the large number of inflammatory markers measured, which may limit statistical power, especially in the smaller LOAD group. We observed no association between the normalized cognitive scores and the different biomarkers after controlling for age and sex, which likely reflects the size and heterogeneity of our cohort as well as differences in data collection context and the cognitive scores used. The inclusion of samples collected at a different center may also be a source of variability in our results. Secondly, for the pathway analysis, we defined differentially expressed proteins in the input based on uncorrected *p*-values, due to the limited number of significant proteins after multiple comparison correction. This may limit comparability with previous studies and makes it challenging to interpret the biological significance of differential protein expression. Thirdly, information about genetic risk factors, like APOE4 status, was not always available. While dominantly inherited AD is rare even in YOAD,[2] APOE status on the other hand may contribute to heterogeneity in our cohort. Previous work has shown that APOE4 carriers had more pronounced increases in microglial neuroinflammation- and phagocytosis-related gene expression than non- APOE4 carriers.[69] Here, in a sub-analysis of the 85 participants with known APOE status, we found no evidence that APOE4 positivity was confounding our main findings. Finally, our cohort included different clinical subtypes of AD in addition to the typical amnestic presentation (which represented 65%–70% of each cohort). Recent studies[70,71] have found no differences in neuroinflammatory processes (microglial specifically) between clinical subtypes of AD (CBS versus classic AD dementia) using either PET-based or immunohistochemistry-based methods. Clinical syndromes also show no differences in amyloid pathology, but differences in tau distribution have been reported, and there have been recommendations to investigate pathophysiological processes in relation to tau, rather than syndrome.[72] In our case, the YOAD cohort did not differ from the LOAD cohort in regard to total tau burden, which was in line with previous studies.[73–75] However, we recognize that our underlying assumption that these AD-positive subjects share the same neuroinflammatory processes across clinical subtypes is a limitation.

In summary, our exploratory proteomic analysis reveals that the inflammatory profile and the relationship between inflammation and neurodegeneration differs in YOAD versus LOAD. Further characterization of the inflammatory markers differentially implicated in these two populations may lead to important insights on the pathophysiology of AD. This work also provides potential targets for the development of biomarker-based patient classification and disease-modifying treatments.

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CONFLICT OF INTEREST STATEMENT

I.J. serves on the scientific advisory board (SAB) of Ankarys Therapeutics, YetiWare, Human Exposome Assessment Platform (HEAP) project. He is on a scientific advisory board for Honeywell Healthcare, Lenovo, Arthritis Society Canada, is a co-founder and CSO for LumiNet Bio Incorporated, and is under the Horizon 2020 Framework Program for Research and Innovation. He has received a server donation from Lenovo and licensed access to the mirDIP database from Cardiatec. B.H. owns stock in Novartis; he serves on the SAB of Dewpoint and has an option for stock. He serves on a scientific advisory board or is a consultant for AbbVie, Alexion, Ambagon, Aprinoia Therapeutics, Arvinas, Avrobio, AstraZenica, Biogen, Bioinsights, BMS, Cure Alz Fund, Cell Signaling, Dewpoint, Latus, Merck, Novartis, Pfizer, Sanofi, Sofinnova, Takeda, TD Cowen, Vigil, Violet, Voyager, WaveBreak. SEA is a paid consultant for Allyx Therapeutics, BioVie, Bob's Last Marathon, Merck, Jocasta Neuroscience, Sage Therapeutics, Sanofi, and Vandria. He has received payments for expert testimony from Foster & Eldredge and ProSelect Insurance Co, and has received grants or contracts from Abbvie, AC Immune, Alzheimer's Association, Athira, Challenger Foundation, Novartis, Seer Biosciences, Venture Well, Chromadex, Ionis Pharmaceuticals, Janssen Pharmaceuticals, the John Sperling Foundation, the NIH, Gatehouse Bio, Eli Lilly/Fortrea, and SuperFluid Dx. M.I. is a paid consultant for BioArctic AB and Eisai Pharmaceuticals. AML receives consulting fees from Abbott, Boston Scientific, Insightec, Medtronic, and Functional Neuromodulation (Scientific Director). MCT has received in-kind funding from Roche. She conducts clinical trials for Biogen, Anavex, Janssen, Novo Nordisk, Merck, Green Valley, UCB. Author disclosures are available in the supporting information.

CONSENT STATEMENT

The study was conducted in accordance with the revised Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Research Ethics Board of the Toronto Western Hospital (University Health Network) and the Mass General Brigham Institutional Review Board (2018P001989). Informed consent was obtained from subjects included in the study.

DIVERSITY, EQUITY, AND INCLUSION

This study included 90 patients with Alzheimer's disease and 26 healthy controls. The population was diverse in regard to gender, with approximately 50% of female subjects in both Alzheimer's disease and healthy control groups. To account for potential differences in socioeconomic status (specifically, differences in education level) and to account for the large age range covered in this study, we used z-scores for the comparison of cognitive impairment. Detailed demographics are reported in Table 1.

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