

Ante Mortem Cerebrospinal Fluid Tau Levels Correlate With Postmortem Tau Pathology in Frontotemporal Lobar Degeneration

David J. Irwin, MD,^{1,2} Alberto Lleó, MD,⁶ Sharon X. Xie, PhD,³
 Corey T. McMillan, PhD,² David A. Wolk, MD,⁴ Edward B. Lee, MD, PhD,⁵
 Viviana M. Van Deerlin, MD, PhD,¹ Leslie M. Shaw, PhD,¹
 John Q. Trojanowski, MD, PhD,¹ and Murray Grossman, MD²

Objective: To test the hypotheses that (1) antemortem cerebrospinal fluid (CSF) tau levels correlate with postmortem tau pathology in frontotemporal lobar degeneration (FTLD) and (2) tauopathy patients have higher phosphorylated-tau levels compared to transactivation response element DNA-binding protein 43 (TDP-43) proteinopathy patients while accounting for Alzheimer's disease (AD) copathology.

Methods: Patients had autopsy-confirmed FTLD with tauopathy (n = 31), TDP-43 proteinopathy (n = 49), or AD (n = 26) with antemortem CSF. CSF tau levels were compared between groups and correlated with digital histology measurement of postmortem tau pathology averaged from three cerebral regions (angular gyrus, mid-frontal cortex, and anterior cingulate gyrus). Multivariate linear regression tested the association of ante mortem CSF tau levels with postmortem tau pathology adjusting for demographics.

Results: Multivariate regression found an independent association of ante mortem CSF phosphorylated tau levels with postmortem cerebral tau pathology in FTLD (Beta = 1.3; 95% confidence interval = 0.2-2.4; $p < 0.02$). After excluding patients with coincident AD-associated tau pathology accompanying sporadic FTLD, we found lower CSF phosphorylated tau levels in the TDP-43 group (median = 7.4pg/ml; interquartile range [IQR] = 6.0, 12.3; n = 26) compared to the tauopathy group (median = 12.5pg/ml; IQR = 10.7, 15.0; n = 23; $Z = 2.6$; $p < 0.01$).

Interpretation: CSF phosphorylated-tau levels are positively associated with cerebral tau burden in FTLD. In vivo detection of AD copathology in sporadic FTLD patients may help stratify clinical cohorts with pure neuropathology in which low CSF phosphorylated-tau levels may have diagnostic utility to distinguish TDP-43 proteinopathy from tauopathy. Autopsy-confirmed samples are critical for FTLD biomarker development and validation.

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Cerebrospinal fluid (CSF) biomarkers of total-tau (t-tau) and phosphorylated-tau (p-tau) have been extensively studied in the context of aging and Alzheimer's disease (AD),¹ where the density of postmortem cortical tau pathology is most closely associated with

antemortem CSF p-tau levels^{2,3} and increased t-tau levels are thought to reflect nonspecific axonal damage and neuronal loss.^{4–6}

Nearly half of all patients presenting with a frontotemporal dementia (FTD) clinical syndrome have

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Address correspondence to Dr David J. Irwin, Frontotemporal Degeneration Center (FTDC), University of Pennsylvania Perelman School of Medicine, Hospital of the University of Pennsylvania, 3600 Spruce Street, Philadelphia, PA 19104. E-mail: dirwin@mail.med.upenn.edu

From the ¹Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, ²Frontotemporal Degeneration Center, Department of Neurology, ³Department of Biostatistics and Epidemiology, ⁴Alzheimer's Disease Center, Department of Neurology, and ⁵Translational Neuropathology Lab, Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; and ⁶Department of Neurology, Institut d'Investigacions Biomèdiques Sant Pau, Hospital de Sant Pau, Barcelona, Spain and Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas, CIBERNED, Barcelona, Spain

neuropathological findings of primary tauopathy consistent with frontotemporal lobar degeneration (ie, FTLD-Tau).⁷ However, the relationship between antemortem CSF t-tau and p-tau with postmortem FTLD tau pathology has not been systematically studied. Indeed, the majority of CSF biomarker studies in FTLD to date have been performed in clinically diagnosed FTD cohorts where >50% of patients may have transactivation response element DNA-binding protein 43 (TDP-43) proteinopathy (FTLD-TDP) or an atypical clinical variant of AD neuropathology.⁷ Furthermore, AD copathology is not uncommon in FTLD and other neurodegenerative disorders, and this secondary AD pathology may influence CSF biomarker levels of tau.^{8,9} Finally, hereditary forms of FTLD may have divergent patterns of pathology,^{10,11} more aggressive disease^{12–14} and additional proteinopathy¹⁵ that could potentially influence CSF biomarkers.⁷ A recent comprehensive review thus indicates considerable variability in reported values of CSF t-tau and p-tau levels in clinical FTD.¹⁶ Several recent studies have examined patients with clinical syndromes highly predictive of molecular etiology or autopsy/genetic confirmed samples and find that there may be diagnostic utility to differentiate FTLD-Tau from FTLD-TDP using a diagnostic cutoff of CSF p-tau or p-tau/t-tau ratio, but it is unclear whether this diagnostic accuracy is driven by lower p-tau or higher t-tau in FTLD-TDP.^{17–19}

Here, we examine a large cohort of autopsy-confirmed patients to examine the relationship between the severity of postmortem cerebral tau pathology and CSF tau biomarkers in FTLD and AD using a validated semiquantitative digital image analysis of histology sections.²⁰ We provide the first direct correlation of CSF p-tau levels with cerebral tau pathology in FTLD. Furthermore, we find that CSF p-tau is lower in FTLD-TDP than FTLD-Tau on group-wise comparisons of FTLD patients with pure sporadic FTLD pathology and show individual patient-level diagnostic specificity for low CSF p-tau, emphasizing that coincident AD neuropathology and mutation status should be taken into account when interpreting CSF biomarkers in FTLD.

Patients and Methods

Patients

Patients were followed clinically at the Penn Frontotemporal Degeneration Center or Alzheimer's Disease Center and autopsied at the Penn Center for Neurodegenerative Disease Research. We identified patients with a primary neuropathological diagnosis of FTLD-Tau (n = 31) or TDP-43 proteinopathy (ie, FTLD-TDP and/or amyotrophic lateral sclerosis [ALS]; n = 49) and a reference group of patients with primary

AD pathology (n = 26) with available ante mortem CSF for analysis (Table 1). Neuropathological examination was performed using established methods²¹ and criteria²² as described. To examine the isolated contribution of AD neurofibrillary tau pathology on CSF biomarkers, we excluded primary AD patients with secondary limbic (ie, amygdala, hippocampal) α -synuclein²³ or TDP-43 pathology²⁴ (n = 5). Braak tau stages were obtained at autopsy using evaluation of the hippocampus and cortical regions²² using p-tau immunohistochemistry (IHC) in FTLD-TDP and AD. In FTLD-Tau patients, sections of the hippocampus were stained with the amyloid-binding dye, Thioflavin-S, as described,²⁵ to distinguish comorbid age-related AD neurofibrillary tangle (NFT) pathology from primary FTLD-tauopathy²⁶ for tau Braak staging by two experienced reviewers (D.J.I., E.B.L.) blinded to CSF data. Both staining methods are considered equivalent for AD neuropathological diagnostic criteria.²²

CSF Analysis

Ante mortem CSF was collected through standardized operating procedures as described⁸. One of two analytical platforms (ie, Innostest enzyme-linked immunosorbent assay [ELISA]; Fujirebio-Europe or INNO-BIA AlzBio 3 xMAP Luminex; Fujirebio-Europe, Gent, Belgium) were used to measure CSF t-tau, p-tau (threonine181), and amyloid-beta1-42 ($A\beta_{1-42}$) as described.⁸ We previously found that absolute values for these analytes are highly correlated between platforms and can be transformed into equivalent units for analysis.^{8,27,28} Thus, we used a validated algorithm⁸ to transform ELISA t-tau and p-tau into equivalent xMAP units for analysis.

Digital Image Analysis of Histology

We used a validated sampling and intensity thresholding method²⁰ to quantify the percent area occupied (%AO) of total p-tau immunoreactivity (AT8²⁹; Fisher Thermo Scientific, Waltham, MA) in an anterior (mid-frontal cortex; MFC), posterior (angular gyrus; ANG), and limbic (anterior cingulate gyrus; CING) region. Because p-tau IHC detects both AD- and FTLD-Tau-associated tauopathy, we performed a subanalysis of FTLD patients after excluding those with AD tau Braak stages B2 or B3, consistent with age-associated tangles extending beyond medial limbic structures,³⁰ to examine the relationship between pure FTLD-Tau pathology and CSF p-tau levels.

All slides in each region were stained in the same batch to reduce "run-to-run" variation. Briefly, digital images were obtained using a Lamina (PerkinElmer, Waltham, MA) slide scanning system at 20 \times . Digital image analysis was performed using Halo digital image software (v1.90; Indica Labs, Albuquerque, NM). We used a vertical-transect method³¹ to sample the longest intact parallel-oriented gray matter (GM) ribbon to reduce bias from over- or under-representation of cortical laminae that preferentially contain FTLD and AD neuropathology.³² We also sampled the largest available deep white matter (WM) area per slide using the rectangular selection tool. A random sampling of 30% of the GM and WM regions selected was performed using 175- μ m tiles, and a validated intensity

TABLE 1. Patient Data

	FTLD-TDP N = 49	FTLD-Tau N = 31	AD N = 26
Sex	M = 24 F = 25	M = 20 F = 11	M = 14 F = 12
Neuropathological subtype	ALS = 10 ALS-FTD = 4 A = 6 B = 15 C = 9 UC = 3	PID = 5 PSP = 10 CBD = 12 FTDP17 = 3 TUC = 1	—
Hereditary mutations	<i>GRN</i> = 8 <i>C9orf72</i> = 12	<i>MAPT</i> = 3	—
PMI	12 (7, 18)	12.5 (6.4,18.3)	12.0 (7.8, 20.0)
Brain weight (g)	1,174.9 (220.0)	1,126.6 (135.3)	1,109.4 (142.2)
Braak tau stage	B0 = 18 B1 = 21 B2 = 07 B3 = 03	B0 = 13 B1 = 14 B2 = 02 B3 = 02	B2 = 02 B3 = 24
CERAD plaque stage	C0 = 37 C1 = 04 C2 = 03 C3 = 05	C0 = 21 C1 = 05 C2 = 03 C3 = 02	C2 = 02 C3 = 24
Age at onset (y)	60.4 (8.8)	59.9 (9.2)	64.5 (11.1)
Age at death (y)	66.6 (9.8)	67.6 (10.0)	73.0 (11.8)^a
Disease duration (y)	6.1 (3.5)	7.8 (4.2)	8.5 (3.5)^a
Age at CSF (y)	63.3 (9.1) N = 49	63.5 (10.0) N = 31	67.9 (10.7) N = 26
Onset-CSF interval (y)	2 (1, 4) N = 49	3 (2, 4) N = 31	2.5 (2, 5.3) N = 26
CSF-death interval (y)	3 (1, 5) N = 49	4 (1, 6) N = 31	5.5 (2, 7)^b N = 26
CSF A β_{1-42} (pg/ml)	226.3 (69.0) N = 40	220.6 (46.4) N = 24	117.6 (37.3)^{a,c} N = 23
CSF τ -tau (pg/ml)	45.5 (29.0, 84.3) N = 49	44.2 (30.0, 67.0) N = 31	98.4 (57.4, 175.8)^{b,c} N = 26
CSF p-tau (pg/ml)	8.5 (6.5, 17.0) N = 47	12.0 (10.8, 15.5) N = 29	36.8 (23.7, 72.9)^{b,c} N = 26

Normally distributed variables are reported as mean (standard deviation) and non-normally distributed variables are reported as median (first quartile, third quartile).

^a $p < 0.05$ compared to FTLD-TDP.

^b $p < 0.01$ compared to FTLD-TDP.

^c $p < 0.01$ compared to FTLD-Tau.

PMI = postmortem interval; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; A β_{1-42} = amyloid-beta1-42; τ -tau = total tau; p-tau = phosphorylated-tau; TDP = transactivation response element DNA-binding protein; M = male; F = female; UC = unclassifiable TDP subtype; ALS = amyotrophic lateral sclerosis (2 patients have ALS with mild cognitive impairment); ALS-FTD = ALS with FTD; PID = Pick's disease; CBD = corticobasal syndrome; PSP = progressive supranuclear palsy; FTDP-17 = frontotemporal dementia with *MAPT* mutation and tauopathy; TUC = tauopathy unclassifiable.

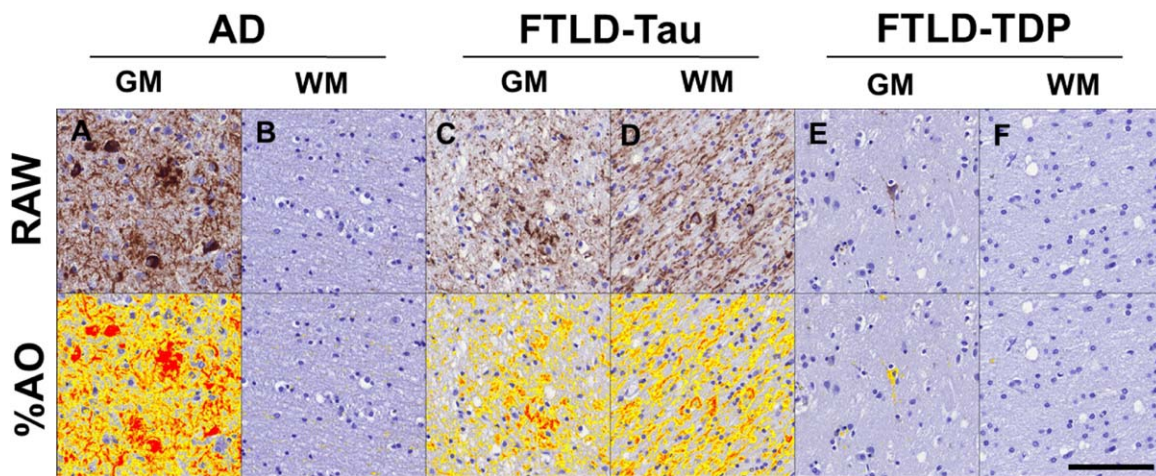


FIGURE 1: Digital image analysis of cerebral tau pathology. Representative photomicrographs depict raw images (top) and digital image analysis thresholding quantification (%AO; bottom, red/orange/yellow overlay) of tau pathology in the mid-frontal cortex for (A,B) AD, (C,D) FTLD-Tau, and (E,F) FTLD-TDP with coincident AD pathology. There is higher overall and GM burden of cerebral tau pathology in AD compared to FTLD-Tau and FTLD-TDP, whereas FTLD-Tau has higher WM tau pathology compared to both groups. AD = Alzheimer's disease; %AO = percent area occupied; FTLD = frontotemporal lobar degeneration; GM, gray matter; WM, white matter.

threshold was applied to quantify all pathological tau (Fig 1) in each random tile. We used the average %AO value from randomly placed tiles for each GM and WM selection per slide. Because FTLD-Tau has a significant burden of WM tau pathology,^{25,33} we added the GM and WM tau %AO in each region and used the average GM + WM sum from the three cerebral regions in each group for comparative analysis (ie, cerebral tau %AO). Cerebral tau %AO measurements were validated through comparison to traditional ordinal rating scores, as we have done previously. There was missing tissue for MFC = 6, ANG = 7, and CING = 6. Cases with missing data ($n = 18$) in one or more of these regions were excluded from total cerebral tau %AO analyses.

Genetic Analysis

DNA was isolated from frozen brain or blood and screened for mutations in *MAPT*, *GRN*, and *C9orf72* based on pedigree analysis for risk of hereditary disease³⁴ using previously described methods.^{25,35}

Statistical Analysis

Variables were examined for normality and one-way analysis of variance or Kruskal-Wallis test were performed across the three neuropathological groups, as appropriate, with planned post-hoc t tests or Mann-Whitney U analyses, respectively, performed between each group. Categorical variables were compared between groups using a chi-square analysis. For correlation and regression models, we used natural log (\ln) transformation to obtain normally distributed variables for analysis. We used Pearson correlation and a multivariate regression model in the FTLD cohort with \ln cerebral tau %AO as the dependent variable and \ln CSF p-tau as the independent variable, adjusting for analytic platform, age, disease duration, and time to death at CSF collection. Model construction was performed using Bayesian information criteria (BIC)³⁶ to derive

the final model (variables that did not improve BIC value were excluded from final model). Demographic variables surviving this model building procedure were used as covariates in the following subsequent analyses. To test the independent association of potential subgroups of FTLD patients that could influence diagnostic accuracy of CSF p-tau levels in FTLD based on previous literature,^{9,16} we first used linear regression analysis with CSF p-tau levels as the dependent variable in the base model, including neuropathological group (FTLD-Tau vs FTLD-TDP), age, and time to death at CSF collection. Our first model examined the independent association of the categorical presence of co-AD tau Braak stages B2-B3 (ie, neocortical AD tau) to those patients with those with pure FTLD pathology (ie, AD Braak tau stages B0-B1). Based on these results, a similar model was performed in the pure FTLD subgroup to test the independent association of the presence of a pathogenic mutation in hereditary FTLD with CSF p-tau levels while covarying for these demographic variables. Receiver operating characteristic curve analysis was performed to test the diagnostic accuracy of CSF p-tau levels.

Missing data were excluded and reported in Table 1. All analyses were performed using two-tailed statistics with $p < 0.05$ using SPSS (v21.0; IBM Corp., Armonk, NY) or STATA software (v12.1; StataCorp LP, College Station TX).

Results

Patient Groups

Table 1 depicts demographics, pathological, and biomarker data for the cohort. Patient groups did not differ in postmortem interval, brain weight, or age at onset. There was a significant difference across groups in age at death (mean difference AD-FTLD-TDP = 6.5, AD-FTLD-Tau = 5.4, FTLD-Tau-FTLD-TDP = -1.1 years; $p = 0.04$) and overall disease duration (mean

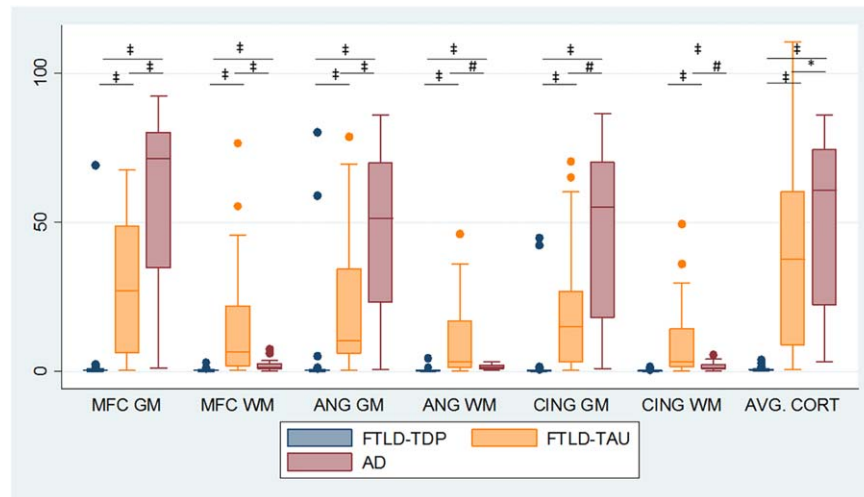


FIGURE 2: Gray and white matter cerebral tau pathology in FTLT-Tau, FTLT-TDP, and AD. Box plots depict values of %AO of tau immunohistochemistry from gray and white matter in each region sampled for each neuropathological group. * $p < 0.05$; # $p < 0.01$; ‡ $p \leq 0.001$. AD = Alzheimer's disease; ANG = angular gyrus; %AO = percent area occupied; AVG CORT = average cerebral tau %AO; CING = anterior cingulate gyrus; FTLT = frontotemporal lobar degeneration; GM, gray matter; MFC = mid-frontal cortex; WM, white matter.

difference AD-FTLT-TDP = 2.4, AD-FTLT-Tau = 0.8, FTLT-Tau-FTLT-TDP = -1.7 years; $p = 0.02$). Planned post-hoc comparisons find AD had a later age at death (mean difference = 6.5 years; $p = 0.02$) and longer disease duration (mean difference = 2.4 years; $p < 0.01$) compared to the FTLT-TDP group.

There was no significant difference between groups with a primary pathologic diagnosis of FTLT-Tau and FTLT-TDP (ie, including cases with coincident secondary AD pathology) for CSF biomarkers and demographics at time of collection (please see below for factors influencing this analysis). The median (range) in duration from CSF collection to death was 3 (<1–12) years for FTLT-TDP, 4 (<1–12) years for FTLT-Tau, and 5.5 (1–10) years for AD. As expected, the AD group had lower $A\beta_{1-42}$ and higher t-tau and p-tau levels compared to both FTLT-Tau (mean difference $A\beta_{1-42} = -102.9\text{pg/ml}$; $p < 0.001$; t-tau median difference = 54.2pg/ml ; $p < 0.001$; p-tau median difference = 24.8pg/ml ; $p < 0.001$) and FTLT-TDP groups ($A\beta_{1-42}$ mean difference = -108.7pg/ml ; $p < 0.001$; t-tau median difference = 52.9pg/ml ; $p < 0.001$; p-tau median difference = 28.3pg/ml ; $p < 0.001$).

Cerebral Tau Burden in FTLT and AD

Reflecting group-wise differences in CSF p-tau levels, comparison of digital image analysis measurement of cerebral p-tau pathology revealed a higher average total cerebral tau %AO in pathological AD compared to cases with primary FTLT-Tau (median difference = 29.9%; $p = 0.03$) and FTLT-TDP pathology (ie, including cases with coincident secondary AD pathology; median difference = 65.9%; $p < 0.001$) and FTLT-Tau also

had higher average total tau %AO than FTLT-TDP (median difference = 36.0%; $p < 0.001$; (Figs 1 and 2). Because FTLT-Tau has considerable WM tau pathology, we also examined GM and WM separately in each region and found that AD had higher average GM tau %AO compared to FTLT-Tau (median difference = 43.8%; $p < 0.01$) and FTLT-TDP (median difference = 64.2%; $p < 0.001$), whereas FTLT-Tau had higher average total cerebral WM tau %AO compared to AD (median difference = 4.1%; $p < 0.01$) and FTLT-TDP (median difference = 5.2%; $p < 0.001$; Figs 1 and 2).

CSF Pathology Associations

In the total cohort, there was a moderate correlation between \ln CSF t-tau and \ln CSF p-tau ($r = 0.5$; $p < 0.001$). Across patient groups, there was a moderate correlation between the \ln average cerebral tau %AO with \ln CSF p-tau ($r = 0.5$; $p < 0.001$; Fig 3A) and less so with \ln CSF t-tau ($r = 0.2$; $p = 0.04$) levels. A subset analysis of the FTLT group alone finds a similar association for the \ln average cerebral tau %AO with \ln CSF p-tau ($r = 0.3$; $p = 0.03$; Fig 3B), but not for \ln CSF t-tau ($r = -0.06$; $p > 0.1$). To account for demographic variables, we used multivariate linear regression in the total FTLT group using \ln average cerebral tau %AO as the dependent variable and found a significant association of \ln CSF p-tau (Beta = 1.3; 95% confidence interval [CI] = 0.2–2.4; $p < 0.02$) when adjusting for demographic covariates (Table 2). A similar model examining \ln CSF t-tau finds no significant association with postmortem tau %AO (data not shown), conferring specificity of ante mortem CSF p-tau for all forms of tau

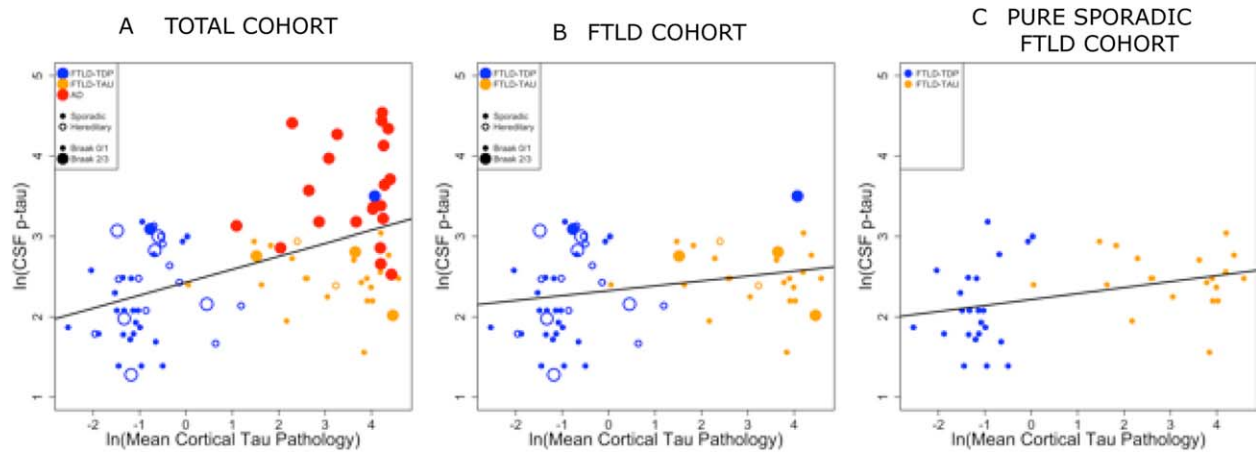


FIGURE 3: Correlation of ante mortem CSF phosphorylated tau measurements with postmortem cerebral tau severity. Scatter plot depicts individual patient data points coded for by primary pathology (blue = FTLD-TDP; orange = FTLD-Tau; red = AD), the presence of hereditary mutations (open circles), and AD tau copathology (large circles = AD tau Braak B2/B3) for natural-log transformed CSF p-tau levels (y-axis) compared to natural-log transformed average cerebral tau %AO pathology measurement (x-axis) in (A) the total FTLD and AD cohort ($r = 0.5$; $p < 0.01$) and (B) the total FTLD cohort ($r = 0.3$; $p = 0.02$) and (C) pure sporadic FTLD cohort ($r = 0.4$; $p = 0.02$) excluding patients with AD copathology or a hereditary mutation. AD = Alzheimer’s disease; %AO = percent area occupied; CSF = cerebrospinal fluid; FTLD = frontotemporal lobar degeneration; p-tau = phosphorylated-tau.

pathology (ie, FTLD-tau and co-incident AD tau in both groups).

A comparison of our entire FTLD cohort (ie, including cases with coincident secondary AD pathology)

revealed a nonsignificant trend for lower CSF p-tau in FTLD-TDP compared to FTLD-Tau (Fig 4A). Because the presence of AD copathology or a pathogenic mutation in the FTLD group appeared associated with higher

TABLE 2. Multivariate Regression Models to Predict Postmortem Cerebral Tau Pathology Table displays optimal multivariate model using natural-log %AO cerebral tau postmortem pathology measurement as the dependent variable and natural-log ante mortem CSF p-tau levels as an independent variable adjusting for demographic features for A) the total FTLD cohort (Model $R^2 = 0.21$; $F_{(3,63)} = 5.6$; $p < 0.01$) and B) the subset analysis of sporadic patients with “pure” (AD Braak tau copathology stage = B0/B1) FTLD (Model $R^2 = 0.28$; $F_{(3,39)} = 5.1$; $p < 0.01$).

A) Total FTLD Cohort			
Variable	Beta (95% CI)	T-Value	p
In CSF p-tau	1.3 (0.2–2.4)	2.6	0.01
Age at CSF collection (y)	–0.1 (–0.1 to –0.01)	–2.4	0.02
CSF collection-death interval (y)	0.2 (0.02–0.4)	2.3	0.03
Intercept	0.7 (–2.7 to 4.1)	0.4	0.7
B) “Pure” Sporadic FTLD Cohort			
Variable	Beta (95% CI)	T-Value	p
In CSF p-tau	2.0 (0.6–3.4)	3.0	<0.01
Age at CSF collection (y)	–0.1 (–0.1 to –0.01)	–2.2	0.04
CSF collection-death interval (y)	0.2 (–0.04 to 0.4)	1.6	0.1
Intercept	–0.2 (–4.8 to 4.4)	–0.1	0.9

CSF = cerebrospinal fluid; p-tau = phosphorylated-tau; FTLD = frontotemporal lobar degeneration; CI = confidence interval; %AO = percent area occupied; AD = Alzheimer’s disease.

TABLE 3. Influence of AD Copathology and Mutation Status on CSF Phosphorylated Tau Levels in FTLD.

Table displays optimal multivariate model using natural-log transformed CSF phosphorylated-tau (p-tau) measurement as the dependent variable to test A) the independent association of the categorical presence of AD copathology (ie, AD Braak tau copathology stage = B2/B3 vs B0/B1) in the total FTLD cohort (Model $R^2 = 0.14$; $F_{(4,71)} = 2.8$; $p < 0.05$) and B) the independent association of the presence of a pathogenic mutation with CSF p-tau measurement in the subset of patients with “pure” (AD Braak tau copathology stage = B0/B1) FTLD (Model $R^2 = 0.20$; $F_{(4,58)} = 3.5$; $p < 0.02$).

C) Total FTLD Cohort			
Variable	Beta (95% CI)	T-Value	<i>p</i>
AD copathology (Braak B2/B3 vs B0/B1)	0.4 (0.04–0.7)	2.3	0.027
FTLD pathology group (FTLD-Tau vs FTLD-TDP)	0.2 (–0.06 to 0.5)	01.54	0.127
Age at CSF collection (y)	0.01 (–0.002 to 0.02)	1.7	0.086
CSF collection-death interval (y)	0.01 (–0.03 to 0.06)	0.6	0.545
Intercept	1.5 (0.6–2.3)	3.6	0.001
D) “Pure” FTLD Cohort			
Variable	Beta (95% CI)	T-Value	<i>p</i>
Mutation status	0.3 (0.004–0.5)	2.0	0.047
FTLD pathology group (FTLD-Tau vs FTLD-TDP)	0.3 (0.1–0.6)	2.9	0.005
Age at CSF collection (y)	0.01 (0.00–0.02)	1.9	0.052
CSF collection-death interval (y)	0.003 (–0.03 to 0.04)	0.2	0.865
Intercept	1.4 (0.6–2.1)	3.5	0.001

AD = Alzheimer’s disease; CSF = cerebrospinal fluid; FTLD = frontotemporal lobar degeneration; TDP = transactivation response element DNA-binding protein; CI = confidence interval.

CSF p-tau levels in individual patient data (Figs 3A and 4A) and previous literature,^{9,16} we first analyzed the association of comorbid AD tau pathology (ie, AD Braak tau stage B2 or B3) with CSF p-tau while covarying for neuropathological diagnosis and demographics. This analysis revealed an independent association of AD copathology with CSF p-tau levels (Beta = 0.4; 95% CI = 0.04–0.7; $p < 0.03$; Table 3A), suggesting that AD copathology can influence CSF p-tau measurements independent of FTLD proteinopathy. As such, the remainder of analyses focused on patients with pure FTLD TDP-43 or tau pathology (ie, minimal AD tau copathology restricted to the medial temporal lobe; AD tau Braak stages B0–B1) and excluded patients with AD Braak tau stages B2 or B3 ($n = 14$; ie, AD tau copathology extending into the neocortex). Group-wise analysis of pure FTLD patients finds a lower CSF p-tau level in the FTLD-TDP group ($n = 35$) compared to the FTLD-Tau group ($n = 25$; median difference = -4pg/ml ; $p < 0.03$; Fig 4B). We

found a higher CSF p-tau/ $A\beta_{1-42}$ ratio in FTLD with AD copathology ($n = 10$) compared to pure FTLD ($n = 53$; median difference = 0.03; $p < 0.04$). To test the ability of CSF to identify AD copathology in FTLD, we excluded patients with CSF p-tau/ $A\beta_{1-42}$ ratio value indicative of AD pathology (ie, >0.1)¹ and found a similar lower CSF p-tau level in FTLD-TDP ($n = 23$) compared to FTLD-Tau ($n = 31$; median difference = -4pg/ml ; $p = 0.03$).

Next, based on our independent patient data (Fig 3A) and significant literature of pathophysiological differences between hereditary and sporadic FTLD,^{10–15} we examined the association of an FTLD-associated pathogenic mutation with ln CSF p-tau using a similar linear regression model in the pure FTLD cohort without AD copathology and found independent association of mutation status (Beta = 0.3; 95% CI = 0.004–0.5; $p < 0.05$) and FTLD-Tau group membership (Beta = 0.3; 95% CI = 0.1–0.5; $p < 0.01$; Table 3B).

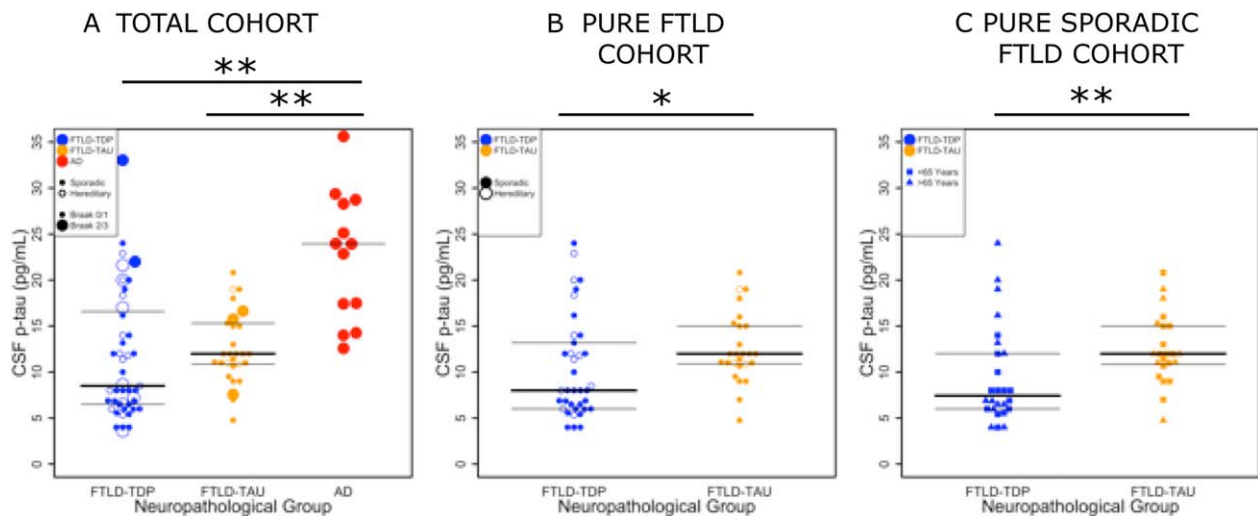


FIGURE 4: CSF p-tau levels in FTLT neuropathological groups with and without coincident AD neuropathology or hereditary disease. Scatter plots depict individual data points in each group coded for the primary pathology (blue = FTLT-TDP; orange = FTLT-Tau; red = AD), presence of AD tau copathology (large circles = AD tau Braak B2/B3), and hereditary mutations (open circles) for CSF p-tau values (pg/ml) in (A) the total FTLT and AD cohort, (B) pure FTLT patients (excluding those cases with coincident AD-associated tau pathology [$n = 14$]), and (C) pure sporadic FTLT additionally excluding those pure FTLT patients with a hereditary mutation ($n = 15$) and data points coded for age <65 years (triangles). Bars represent median and interquartile range values. $*p < 0.02$; $**p < 0.01$. AD = Alzheimer's disease; CSF = cerebrospinal fluid; FTLT = frontotemporal lobar degeneration; p-tau = phosphorylated-tau.

Given that both AD copathology and pathogenic mutation status may obscure meaningful comparisons between group in the majority of FTLT patients with pure pathology and sporadic disease (Fig 4A), the following evaluation of diagnostic accuracy and group-wise comparisons focused on sporadic patients with pure FTLT pathology ($n = 49$). First, re-examination of the relationship between ante mortem ln CSF p-tau and ln postmortem cerebral tau pathology in the subset of pure FTLT patients with sporadic disease finds a stronger correlation ($r = 0.4$; $p = 0.02$; Fig 3C). Moreover, using linear regression to adjust for demographics, there was also a significant association of ln CSF p-tau with ln postmortem measurement of cerebral tau pathology (Beta = 1.7; 95% CI = 0.2–3.1; $p = 0.02$; Table 2B), confirming that CSF p-tau directly relates to postmortem tau pathology in pure sporadic FTLT alone. Next, a focused subset group-wise analysis of pure sporadic FTLT patients finds a lower CSF p-tau level in the FTLT-TDP group ($n = 26$) compared to the FTLT-Tau group ($n = 23$; median difference = -3.3 pg/ml; $p < 0.01$; Fig 4C).

In an exploratory analysis of diagnostic accuracy of CSF p-tau in our pure sporadic FTLT cohort, we found that the values below the optimal cut point of 10.3pg/ml had 89.8% specificity and 69.2% sensitivity for pure sporadic FTLT-TDP compared to the combined AD and pure sporadic FTLT-Tau group (AUC = 0.85; 95% CI = 0.76–0.94; $p < 0.001$) and 78.3% specificity and

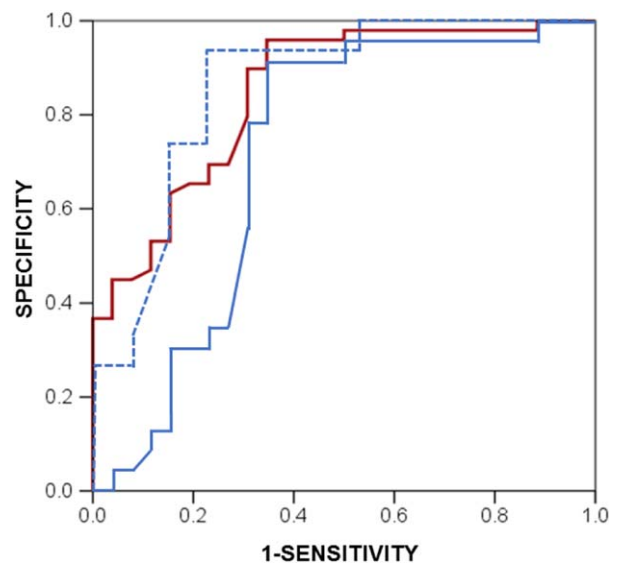


FIGURE 5: Diagnostic accuracy to distinguish FTLT-TDP in sporadic patients without AD copathology. Receiver operating characteristic curve depicts the diagnostic accuracy of CSF p-tau levels for sporadic FTLT-TDP patients with pure pathology (ie, AD tau Braak B0/B1; $n = 26$) compared to the combined AD and pure sporadic FTLT-Tau group ($n = 49$; red solid line; AUC = 0.85; 95% CI = 0.76–0.94; $p < 0.001$) and pure sporadic FTLT-Tau group alone ($n = 23$; blue solid line; AUC = 0.72; 95% CI = 0.57–0.87; $p < 0.01$). Subanalysis of pure sporadic FTLT cases aged <65 years finds increased diagnostic accuracy using CSF p-tau to differentiate FTLT-TDP from FTLT-Tau (AUC = 0.86; 95% CI = 0.71–1.0; $p = 0.01$; blue dashed line). AD = Alzheimer's disease; AUC = area under the curve; CI = confidence interval; CSF = cerebrospinal fluid; FTLT = frontotemporal lobar degeneration; p-tau = phosphorylated-tau.

66.4% sensitivity for pure sporadic FTLD-TDP compared to the pure sporadic FTLD-Tau group alone (AUC = 0.72; 95% CI = 0.57–0.87; $p < 0.01$; Fig 5). Examination of the pure sporadic FTLD-TDP false-negative patients (ie, CSF p-tau >10.3) found that they were older at the age of CSF collection (mean = 70.8 \pm 6.6) compared to true positive FTLD-TDP patients (ie, CSF p-tau < 10.3; mean = 60.9 \pm 10.9; $p = 0.01$), whereas the frequency of clinical ALS was higher in the true-positive FTLD-TDP patients (8 of 18; 44%) than false-negative FTLD-TDP patients (2 of 8; 25%); this did not reach significance ($\chi^2 = 0.9$; $p = 0.4$). Restriction of CSF p-tau diagnostic accuracy assessment to pure sporadic FTLD patients younger than 65 ($n = 28$) found 93.3% specificity and 77.9% sensitivity using a CSF p-tau level cutoff of 9.76pg/ml (AUC = 0.86; 95% CI = 0.71–1.0; $p = 0.01$; Fig 5).

Discussion

Here we provide, to our knowledge, the first direct assessment of the relationship between ante mortem CSF p-tau levels and postmortem tau pathology in FTLD. Using a novel, sensitive digital histology method, we found that ante mortem CSF p-tau directly correlates with postmortem cerebral tau pathology in FTLD (both including and excluding comorbid AD neurofibrillary tauopathy) while adjusting for demographics at the time of CSF collection. Moreover, after exclusion of patients with coincident AD neuropathology (i.e. Braak B2–B3) and those with mutations, patients with pure sporadic FTLD-TDP had significantly lower CSF p-tau levels than pure sporadic FTLD-Tau pathology (Fig 4B,C) with individual-patient level diagnostic accuracy of high specificity (>78–89%) and moderate sensitivity (66–78%). These data highlight the importance of autopsy-confirmed samples in the study of biomarkers of FTLD, and suggest a strategy by which traditional CSF analytes may contribute to diagnosis and stratification in disease-modifying clinical trials.

CSF p-tau, but not CSF t-tau, was closely associated with postmortem tau deposition (Fig 3), supporting the notion that p-tau better reflects tau pathology, whereas t-tau elevations reflect nonspecific neuronal injury.^{4–6} The correlation between CSF p-tau and t-tau levels ($r = 0.5$; $p < 0.01$) in our total cohort was similar to a previous report of a mixed cohort of FTLD, AD, and controls ($r = 0.67$; $p < 0.001$),³⁷ but lower than a large clinical AD series ($r = 0.77$ – 0.88 ; $p \leq 0.001$).³⁸ This discrepancy could be attributed to sample size, analytical factors, or differences between AD and FTLD tau pathology. Indeed, our digital pathology analysis found increased total cerebral tau pathology in the AD group

compared to FTLD-Tau, and minimal comorbid AD tau pathology in the majority of FTLD-TDP (Fig 2), reflecting the group-wise comparisons of CSF p-tau between these groups when accounting for co-AD pathology in FTLD (Fig 4A). As expected, FTLD-Tau had higher WM pathology than AD (Fig 2). AD tauopathy is largely contained within neuropil threads³⁹ with minimal WM tau pathology.³³ FTLD-Tau also has varying degrees of neuronal and glial GM tau pathology, but this was not as severe as AD GM pathology in our quantitative assessment. Several important distinctions exist between FTLD-associated tauopathy and AD-associated NFTs. These include ultrastructural features⁴⁰ and the presence of conformational tau epitopes⁴¹ and amyloid-binding dye reactivity (eg, Thioflavin-S) in mature AD NFT tangles³³ that are largely absent in FTLD-Tau pathology.^{26,42} Finally, AD ghost NFTs remain after neuron loss,⁴³ whereas ghost pathology is largely absent in FTLD-Tau.⁴⁰ Thus, further study is needed on specific forms of pathological tau in the CSF of AD and FTLD patients.

These biochemical and histochemical differences between FTLD- and AD-associated tauopathy notwithstanding, we found a correlation in the amount of all cerebral tau %AO with ante mortem p-tau levels in CSF across all patient groups and within FTLD. Longitudinal data characterizing change in CSF tau levels in FTLD are lacking, and the few studies of serial CSF collections in AD find variation between individuals in longitudinal change⁴⁴; thus, the timing of CSF collection in the course of disease for our AD group may have influenced results. To account for variance in the timing of CSF collection, we performed a multivariate regression model to adjust for this and other demographics (see Table 2). Further study is needed to fully establish the longitudinal dynamic profile of CSF biomarkers in AD and FTLD; however, based on our data, ante mortem CSF p-tau levels appear to be predictive of the severity of overall FTLD-associated tauopathy.

Although we cannot be certain of the underlying neuropathology in previous clinical FTD patient series, up to 20% of all clinical FTD patients may have primary AD neuropathology,⁷ and this may be even higher in patients with primary progressive aphasia.⁴⁵ Furthermore, coincident AD neuropathology is not uncommon in FTLD,⁹ necessitating autopsy-confirmed samples in biomarker studies. Indeed, in our current cohort, we found 14 FTLD patients with AD-associated tau tangles extending into the neocortex (ie, Braak stages B2–B3). Consequently, we discovered that patients with secondary AD copathology influenced the interpretation of group-wise comparisons of CSF p-tau levels in FTLD (Fig 4A;

Table 3). When we excluded cases with Braak tau stages B2 and B3, consistent with moderate-to-severe AD pathology,²² we found a significant difference in CSF p-tau levels between autopsied FTLD-Tau and FTLD-TDP groups (Fig 4B). Indeed, FTLD patients with AD copathology often have similar CSF p-tau and $A\beta_{1-42}$ levels to AD patients.⁹ In an exploratory analysis, we found a similar group-wise difference in CSF p-tau levels between FTLD-Tau and FTLD-TDP after excluding patients with pathological levels of CSF p-tau/ $A\beta_{1-42}$ ratio,¹ suggesting an iterative evaluation of CSF biomarkers to first detect and exclude AD copathology before interpretation CSF p-tau levels may be useful to distinguish FTLD-Tau from FTLD-TDP in living patients. We did not detect a significant association of CSF p-tau with tau pathology within the FTLD-Tau group alone (data not shown); however, we were limited by ceiling effects for the very high tau pathologic burden and lack of very rare presymptomatic autopsy patients with low levels of tau pathology in the FTLD-Tau group, precluding reasonable statistical assessment with our relatively small sample size. These data suggest that in vivo screening for AD neuropathology, using methods such as emerging amyloid-beta⁴⁶ and tau⁴⁷ imaging ligands, as well as CSF $A\beta_{1-42}$, before assessing CSF p-tau could potentially be useful to characterize clinical FTD cohorts and aid in the interpretation of CSF biomarkers for clinical trials.

We also found an independent association of mutation status with increased CSF p-tau levels in FTLD after exclusion of AD copathology (see Table 3). Focused study of CSF biomarkers in hereditary FTLD are rare and often lack autopsy confirmation.¹⁶ Thus, the exact nature of our association of hereditary FTLD with CSF p-tau is unclear; however, a large body of pre-existing literature¹⁰⁻¹⁵ suggests altered underlying pathophysiology compared to sporadic disease, which could contribute to altered CSF biomarker levels.⁷ Most hereditary patients in our cohort had an FTLD-TDP-associated mutation, and we cannot evaluate the association of specific molecular etiologies within the hereditary FTLD subcohort in the current study. Furthermore, these mutations are predictive of molecular pathology⁷ and can be detected clinically through pedigree analysis.³⁴ Therefore, we excluded hereditary patients from our diagnostic accuracy assessment (Fig 5), which was performed to provide proof of concept for the clinical use of CSF p-tau levels in sporadic FTLD. Using our pure sporadic FTLD cohort, we did find high specificity and moderate sensitivity to distinguish FTLD-TDP (Fig 5). We found that some pure FTLD-TDP cases had levels of CSF p-tau similar to that of FTLD-Tau or AD (Fig 4C), reflecting the moderate

sensitivity of our optimal cut point. These patients were older, on average, compared to true-positive FTLD-TDP patients with CSF p-tau below our diagnostic cut point, and focused analysis in pure sporadic FTLD patients younger than 65 at the time of CSF collection found increased diagnostic accuracy (Fig 5). Thus, we provide novel data using rare autopsy samples to demonstrate feasibility for diagnostic use of CSF p-tau measurement in the majority of FTLD patients who are young at onset with pure pathology and sporadic disease. Indeed, ~70% of all clinical FTD has an age of onset <65 years.⁴⁸ Because our focus was on the relationship between ante mortem CSF p-tau to postmortem FTLD tau pathology, we did not include a replication cohort and autopsy-confirmed CSF data are extremely rare; however, previous studies consisting largely of living patients with clinical phenotypes predictive of molecular pathology in FTLD⁷ found a similar or higher performance of low CSF p-tau or ratio of p-tau to t-tau to differentiate FTLD-TDP from FTLD-Tau.¹⁷⁻¹⁹ Our pure sporadic FTLD-TDP group included patients with clinical ALS with varying levels of cognitive impairment (see Table 1), which could have influenced our findings; however, the pure sporadic ALS/ALS-FTLD patients were younger than pure sporadic FTLD-TDP patients without clinical ALS by an average of ~14 years (data not shown), so we cannot dissociate the effects of aging and clinical ALS in the current FTLD-TDP autopsy sample. We previously found that nonautopsied ALS patients had lower p-tau levels and lower p-tau/t-tau ratio, but similar t-tau levels, compared to FTLD-Tau and controls,¹⁷ whereas others find similar p-tau levels and higher CSF t-tau levels in ALS compared to FTLD-TDP or controls.^{37,49} Furthermore, we also found similar levels of CSF t-tau (see Table 1) and the ratio of p-tau/t-tau (data not shown) between the FTLD-Tau and FTLD-TDP group, whereas others have found higher CSF t-tau in FTLD-TDP compared to FTLD-Tau.^{18,19} Lack of autopsy data and differences in demographics and mutation status may contribute to these discrepancies between studies. Indeed, we provide here novel tissue validation for CSF p-tau, and not t-tau, for tau pathology in FTLD. Thus, we contend that low CSF p-tau may be associated with TDP-43 proteinopathies, that are characterized by very low tau pathology, especially in younger patients. Further work using prospective autopsy-confirmed FTLD with homogenous genetic backgrounds will help elucidate the complex interactions of CSF tau and underlying molecular neuropathology in FTLD and replicate diagnostic accuracy before clinical use of CSF p-tau to identify TDP-43 proteinopathies.

Several additional limitations to this study should be kept in mind when considering our data. First, referral bias of atypical or aggressive disease for an autopsy cohort in a tertiary center could limit generalization for clinical use in the general dementia clinic population. We did not include normal control data because the focus of this work was on autopsy-confirmed samples and CSF samples from autopsy-confirmed controls are exceedingly rare. Indeed, the high-prevalence of AD-related pathology in the aging population, even in the presence of normal cognition,⁵⁰ likely would influence CSF p-tau levels based on our quantitative pathology data here, necessitating autopsy-confirmed samples to exclude control patients with presymptomatic AD tau pathology and obtain true normative nonpathogenic levels of CSF t-tau and p-tau.

With these caveats in mind, we provide here novel experimental data to suggest that low CSF p-tau levels may be useful as a biomarker to differentiate FTLD-TDP from FTLD-Tau in patients with pure sporadic FTLD pathology. There is need for FTLD-specific biomarkers that could be used in conjunction with CSF p-tau to help predict underlying neuropathology in clinical FTD, which is urgently needed for clinical trials.

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Author Contributions

Concept and design (D.J.I., A.L., C.T.M., and M.G.); data acquisition and analysis (D.J.I., A.L., S.X.X., D.A.W., E.B.L., V.M.V., L.M.S., J.Q.T., and M.G.); and drafting the manuscript and figures (D.J.I., A.L., C.T.M., S.X.X., and M.G.).

Potential Conflicts of Interest

Nothing to report.

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