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ORIGINAL ARTICLE

Tau Isoform Profile in Essential Tremor Diverges From Other Tauopathies

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Abstract

Patients with essential tremor (ET) frequently develop concurrent dementia, which is often assumed to represent co-morbid Alzheimer disease (AD). Autopsy studies have identified a spectrum of tau pathologies in ET and tau isoforms have not been examined in ET. We performed immunoblotting using autopsy cerebral cortical tissue from patients with ET (n = 13), progressive supranuclear palsy ([PSP], n = 10), Pick disease ([PiD], n = 2), and AD (n = 7). Total tau in ET samples was similar to that in PSP and PiD but was significantly lower than that in AD. Abnormal tau levels measured using the AT8 phospho-tau specific (S202/T205/S208) monoclonal antibody in ET were similar to those in PSP but were lower than in PiD and AD. In aggregates, tau with 3 microtubule-binding domain repeats (3R) was significantly higher in AD than ET, while tau with 4 repeats (4R) was significantly higher in PSP. Strikingly, the total tau without N-terminal inserts in ET was significantly lower than in PSP, PiD, and AD, but total tau with other N-terminal inserts was not. Monomeric tau with one insert in ET was similar to that in PSP and PiD was lower than in AD. Thus, ET brains exhibit an expression profile of tau protein isoforms that diverges from that of other tauopathies.

Key Words: Alzheimer disease, Essential tremor, Neurodegeneration, Neurofibrillary tangle, Pick disease, Progressive supranuclear palsy, Tau isoform, Tauopathy.

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INTRODUCTION

Essential tremor (ET) is a progressive neurological disease characterized by kinetic tremor, its hallmark feature, but other types of tremor are often also present (1). The prevalence of ET is estimated to be 4% (age ≥40 years) and exceeding 20% in advanced age (>90 years) (2). Although the etiology of the disease is poorly understood, involvement of susceptibility genes and/or environmental factors has been proposed (3, 4).

An increasing number of independent studies have demonstrated that ET patients have poorer cognitive performance and are more susceptible to the development of progressive and severe cognitive impairment than age-matched controls without ET (5–7). In several population-based studies, both the odds and risk of dementia are elevated in ET cases compared to age-matched controls indicating that ET is a risk factor for dementia (8, 9). The pathophysiology of ET is not fully understood but a cluster of neurodegenerative morphological changes have been identified in the cerebellum, predominantly centered in/around Purkinje cells (10, 11). Building on earlier work linking ET to possible tau dysregulation (12–16), a recent study found greater tau tangle pathology in the neocortex of non-demented ET patients compared to non-demented controls, implying that ET might cause dysregulation of tau proteostasis (17).

Tau is a highly abundant microtubule (MT)-associated protein found in neuronal and glial cells (18). One of its best described functions is to facilitate MT assembly (19). In the human brain, tau predominantly exists as 6 isoforms derived from alternative pre-mRNA splicing of exons 2, 3, and 10 (20). Alternative splicing of exons 2 and 3 results in isoforms with 0, 1, or 2 inserts of the exon-derived sequences in the N-terminal region (0N, 1N, 2N), while alternative splicing of exon 10 yields tau with 3 or 4 MT-binding repeats in the C-terminal MT-binding domain (3R, 4R). In the adult human brain, the overall ratio of 3R and 4R tau isoform levels is roughly equal (21), although this ratio can change in different brain regions and under various pathological conditions (22). Autosomal dominant mutations in splice sites near exon 10, usually causing increased inclusion of this exon and an increase in 4R isoforms, are causal in a subset of patients with frontotemporal lobar degeneration (23). The 17q21.31 *MAPT* H1 haplotype, a large structural genomic inversion and common risk factor for sporadic tauopathy may also be associated with increased expression of 4R tau isoforms (24), but this is controversial (25). The functional consequences of the resultant imbalance in tau isoforms that lead to neurodegeneration remain unclear but 4R tau is more prone to aggregate and may be more toxic than 3R tau (26). How tau isoform imbalances may induce neurodegeneration is unclear. 4R tau isoforms show greater affinity for MTs and increased MT assembly compared to 3R tau in vitro (27, 28). Although the functions of the N-terminal inserts are not well understood, 0N, 1N, and 2N isoforms exhibit distinct subcellular localization and there are also regional differences in the expression of these isoforms in the brain (29, 30). Co-immunoprecipitation studies using mouse brain lysate demonstrate that 0N, 1N, and 2N isoforms differentially interact with distinct sets of proteins (31).

Given the central role of imbalances in tau isoforms in disease mechanisms, their relative abundance in brain tissue serves as a valuable diagnostic feature, marking involvement of specific tauopathic processes (32). Alzheimer disease (AD), chronic traumatic encephalopathy, and primary age-related tauopathy (PART) are characterized by mixed accumulation of both 3R and 4R tau (33–35). 4R predominant tauopathies include progressive supranuclear palsy (PSP), corticobasal degeneration, and argyrophilc grain disease (36–40). Pick disease (PiD), while very rare, is the only 3R predominant tauopathy and its existence raises the possibility that a selective toxicity of 4R is not the only pathological trigger, and that imbalances in tau isoforms alone are pathogenic. Importantly, tau isoforms have not been directly measured in ET, which is associated with tau accumulation and dementia.

Here, we conducted an exploratory study, comparing the tau isoform expression profile in a cohort of ET patients compared to patients with other tauopathies to ascertain the extent to which the cortical tau pathology differs from these other diseases. This was performed by measuring the levels of all 6 brain enriched tau isoforms, including monomeric and oligomerized forms, from postmortem brain tissue from a cohort of 13 ET postmortem brains and then comparing the results to those from patients with AD and 2 amyloid-independent primary tauopathies, PSP and PiD.

MATERIALS AND METHODS

Subjects and Tissue Samples

Fresh frozen postmortem ET brain tissue (temporal cortex, Brodmann area 37) was obtained from the Essential Tremor Centralized Brain Repository at Columbia University (New York, NY). These donors were enrolled prospectively and followed with motor and cognitive assessments, with 10 of 13 also enrolled in an ongoing longitudinal, prospective study of cognitive function in ET (Clinical Pathological Study of Cognitive Impairment in Essential Tremor, NINDS R01NS086736) which has been described elsewhere (41, 42). ET diagnoses were assigned by a senior movement disorder neurologist (E.D.L.) utilizing 3 sequential methods (43). Briefly, the clinical diagnosis of ET was initially assigned by treating neurologists, and secondly, confirmed by E.D.L. using questionnaires, review of medical records, and review of Archimedes spirals. Third, a detailed, videotaped, neurological examination was performed, action tremor was rated, and a total tremor score assigned (range: 0–36 [maximum]). Combined with the questionnaire data, the final diagnosis of each ET case was re-examined, using previously published diagnostic criteria, which have been shown to be both reliable and valid (43). None of the ET cases had a history of traumatic brain injury, exposure to medications with associated cerebellar toxicity (e.g. chemotherapeutic agents) or heavy ethanol use (43). The next-of-kin provided written consent for participation and brain donation. Institutional Review Board approval for collection of clinical data was approved at Yale University and Columbia University Medical Center (CUMC). Diagnoses of mild cognitive impairment (MCI) and dementia among the ET cases were assigned by review of cognitive assessment data by a neuropsychologist (S.C.).

For ET cases, the neuropathological workup was performed at the Essential Tremor Centralized Brain Repository. Frozen tissues (dorsolateral prefrontal cortex) from cases with PSP and PiD were obtained from University of California San Diego, University of Pittsburgh, Emory University, and University of California Irvine. AD cases were obtained from the NeuroBioBank (University of Maryland). The cases represented a convenience sample with available tissue; ET cases were further selected based on the presence of sufficient data on cognitive status. All methods were carried out in accordance with the relevant guidelines, laws and regulations. AD neuropathological assessments were performed using the current consensus guidelines (44–47).

Immunohistochemistry

Portions of frozen tissue were thawed and fixed in 10% neutral-buffered formalin, paraffin embedded, cut at 4–5 μm, placed on charged slides, and baked overnight at 70°C. Immunohistochemistry (IHC) was performed on a Ventana Benchmark XT (Roche Diagnostics, Indianapolis, IN). Antigen retrieval was done using CC1 buffer (Tris/Borate/EDTA buffer, pH 8.0–8.5; Roche Diagnostics, Indianapolis, IN) for 1 hour followed by primary antibody incubation for 30 min (clone AT8, 1:1000, Invitrogen/Thermo Fisher Scientific, Waltham, MA). Slides were imaged using an Olympus BX40

brightfield microscope with an Olympus DP27 camera and CellSens software (Olympus, Center Valley, PA).

Biochemical Studies

Western blotting was performed as previously described (48). Briefly, fresh-frozen brain tissue was homogenized with a glass-Teflon homogenizer at 500 rpm in 10 volumes (wt/vol) of ice-cold tissue homogenization buffer containing 20 mM Tris pH 7.4, 250 mM sucrose, 1 mM EDTA, 1 mM EGTA, and Halt protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). For each sample, 30 µg of protein were boiled in Laemmili sample buffer (Bio-Rad, Hercules, CA) for 5 minutes, run with tau protein ladder (rPeptide, Watkinsville, GA) on 10% PROTEAN TGX Precast Gels (Bio-Rad), blotted to nitrocellulose membranes, and stained with anti-Tau (clone HT7, 1:3000, Invitrogen/Thermo Fisher Scientific), anti-pTau (clone AT8, 1:1000, Invitrogen/Thermo Fisher Scientific), anti-3R tau (clone 8E6/C11, 1:2000, Sigma-Aldrich, St. Louis, MO), anti-4R tau (clone 1E1/A6, 1:500, Sigma-Aldrich), anti-0N tau (clone EPR21726, 1:1000, Abcam, Cambridge, UK), anti-1N tau (clone 4H5.B9, 1:1000, Biolegend, San Diego, CA), anti-2N tau (clone 71C11, 1:1000, Biolegend), and anti-GAPDH (clone 6C5, 1:20000, Abcam) (Table 1). HRP-labeled secondary anti-mouse or rabbit antibody (1:20000, Vector Labs, Burlingame, CA) was detected by SuperSignal West Femto Maximum Sensitivity Substrate or Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific). Chemiluminescence was measured in an LAS-4000 Intelligent Dark Box imager (Fuji Film, Valhalla, NY), and relative optical densities (A.U., arbitrary units) were determined with AlphaEaseFC software, version 4.0.1 (Alpha Innotech, San Leandro, CA), normalized to total protein loaded (GAPDH). Signals above 75 kDa were measured as oligomers, from 45 kDa to 75 kDa as monomers, and 45 kDa and above or combined monomeric and oligomeric as total as previously described (49).

Statistics and Data Analysis

Immunohistochemical AT8-positive p-tau burden was assessed in a semiquantitative manner. For mild cases, rare positive was observed on less than 10% of the stained tissue fragments. For tissues categorized as moderate, the preparation had significantly more than mild cases but did not exceed 50%. Severe cases had staining on a majority of the fragments (>50%). For biochemical analyses, the 13 ET cases were divided into the following 2 groups based on biochemical AT8 levels, ET (high AT8), and ET (low AT8). A cutoff value of low AT8 levels obtained from immunoblots was set to 10 since the highest value was 9 in the cases with negative p-tau burden assessed by IHC. Given this heterogeneity of the presence of p-tau aggregates among our ET samples, and the exploratory nature of this study, we analyzed these 2 groups separately and additionally as a combined group. Densitometry data were compared using one-way ANOVA with post-hoc Tukey's HSD test for pair-wise comparisons of groups. The vast majority of data elements were normally distributed; hence, a parametric approach was used for data analysis. Using correlation coefficients, we also assessed whether age was associated with values of each tau isoform variable. Statistical analysis and graph production were done in GraphPad Prism (GraphPad Software, San Diego, CA).

RESULTS

In this study, we compared the tau pathology, including burden of abnormal AT8-positive phosphorylated tau and isoform ratios, in the brains from 13 ET patients with 10 PSP, 2 PiD, and 7 AD (Table 2). These ET cases were a convenience sample derived from the Essential Tremor Centralized Brain Repository. The average and median age of death for the ET cases was 90.2 and 90 years old, respectively (range: 83–99) with 2 males and 11 females. Six were demented, 4 had MCI, and 3 were cognitively normal (Table 3). Following comprehensive histopathological assessments for neurodegenerative disease, 8 had AD neuropathologic change as the primary neuropathological dementia diagnosis, 2 had hippocampal sclerosis, 1 had PART, 1 had mild Lewy body pathology (mild and involving the dorsal motor nucleus of the vagus and substantia nigra, but sparing the locus coeruleus and therefore not following a neuropathological stage of Parkinson disease), and the final had corticobasal degeneration. The latter 2 cases had ET for 23 and 58 years, respectively, and neither

TABLE 1. Antisera

Antigen	Clone	Source	Dilution	
			WB	IHC
Total tau	HT7	Invitrogen	1:3000	—
p-tau	AT8	Invitrogen	1:1000	1:1000
3R tau	E6/C11	Sigma-Aldrich	1:2000	—
4R tau	1E1/A6	Sigma-Aldrich	1:500	—
0N tau	EPR21726	Abcam	1:1000	—
1N tau	4H5.B9	Biolegend	1:1000	—
2N tau	71C11	Biolegend	1:1000	—
GAPDH	6C5	Abcam	1:20000	—

IHC, immunohistochemistry; WB, Western blot.

TABLE 2. Patient Data

	ET	PSP	PiD	AD
n	13	10	2	8
Age of death (years)				
50–59	0	1	0	0
60–69	0	3	1	0
70–79	0	1	1	0
80–89	6	4	0	7
90–99	7	0	0	0
Unreported	0	1	0	0
Average	90.2	72.8	71	82.6
Median	90	78	71	81
Sex (male/female)	2/11	3/7	1/1	6/1

AD, Alzheimer disease; ET, essential tremor; PiD, Pick disease; PSP, progressive supranuclear palsy.

TABLE 3. Tau and Other Pathologies in Essential Tremor

Age	Sex	Congnitive Status	1° NP Dx	ADC			p-Tau (AT8) Burden*	
				Thal Phase	Braak NFT	CERAD	IHC	WB (AU)
99	F	Dementia	HPS	III	V–VI	0	++	12.6
89	F	Dementia	ADC	IV–V	V–VI	+++	+++	45.6
90	F	Dementia	ADC	III	V–VI	++	++	18.7
90	F	Dementia	ADC	IV–V	V–VI	++	+++	35.1
85	F	Dementia	ADC	IV–V	V–VI	+++	++	8.0
91	F	Dementia	HPS	III	III–IV	+++	+	8.2
94	F	MCI	ADC	III	III–IV	++	+++	11.8
94	F	MCI	CBD	0	V–VI	0	++	8.6
87	M	MCI	LBD	0	III–IV	0	0	7.0
94	M	MCI	PART	I–II	III–IV	0	0	7.4
83	F	Normal	ADC	III	V–VI	++	+	7.9
89	F	Normal	ADC	III	III–IV	+	0	8.5
88	F	Normal	ADC	III	III–IV	0	0	9.0

*Temporal cortex (Brodmann area 37).
ADC, Alzheimer disease neuropathologic change; AU, arbitrary units; CBD, corticobasal degeneration; CERAD, Consortium to Establish a Registry for Alzheimer's Disease neuritic plaque burden; HPS, hippocampal sclerosis; IHC, immunohistochemistry; LBD, Lewy body disease; MCI, mild cognitive impairment; NFT, neurofibrillary tangle; 1° NP, Dx primary neuropathological diagnosis; PART, primary age-related tauopathy; WB, Western blot.
0, none; +, mild; ++, moderate; +++, severe.

exhibited parkinsonism during life. These findings are consistent with the previous findings of additional heterogeneous neurodegenerative pathologies in elderly ET cases.

We started by performing an immunohistochemical study to assess our frozen ET tissue samples, which were from the contralateral hemisphere to that which was previously evaluated histologically, to confirm and document the presence of the AT8 phosphoepitope (p-tau) and measure their burden. By immunohistochemistry, we observed a varying burden of p-tau, with some ET cases completely devoid of p-tau pathology or with only scattered thread pathology (Fig. 1A, B; Table 3). These findings correlated well with the neuropathological assessments performed on fixed tissues derived from the contralateral hemisphere. Other ET cases had more severe p-tau burden, with robust neuronal and glial accumulation (Fig. 1C, D; Table 3).

Next, we used quantitative immunoblot analysis on the same samples to assess the total and pathological tau burden. The ET cases, including those with dementia, had significantly lower total tau than the AD cases (Fig. 1E, F). Although not statistically significant, total tau levels were numerically higher in ET cases that were cognitively normal versus those with MCI and dementia (Fig. 1G). Yet, the ET normal and dementia cases did have significantly less total tau than the AD group. With respect to p-tau, we observed a similar variability to that which we saw with our immunohistochemical analysis, with negligible to low levels in some of the ET cases and high in others (Fig. 1H, I; Table 3). The highest levels of p-tau were seen in AD and PiD. The levels of total tau and p-tau were more variable in the ET and PSP groups, which were not significantly different. The levels of p-tau in ET cases with MCI and dementia were higher than ET with normal cognitive status (Fig. 1J). Given this heterogeneity in the presence of p-tau aggregates among our ET samples, the 13 ET cases were dichotomized based on biochemical AT8 levels, ET (high AT8), and ET (low AT8). The groups were analyzed both separately and combined. Biochemical AT8 levels were similar to the semiquantitative AT8-positive assessments by immunohistochemistry (Table 3).

Burden of AT8-reactive phosphorylated tau is a robust and reproducible marker of pathology, but it is less useful for diagnostic subclassification on its own. Therefore, we assessed differences in tau isoforms derived from alterative pre-mRNA splicing of exon 10, which reflects divergent underlying pathology, by performing quantitative immunoblots using isoform-specific antisera that recognize 3R or 4R isoforms (Fig. 2A, B). In each sample, we assessed the total levels of the isoforms as well as monomeric and high molecular weight assemblies over 75 kDa ("oligomers"). Total, monomeric, and oligomeric 3R levels in ET were not different from those in PSP and PiD, but significantly lower in AD (Fig. 2C–E). Total, monomeric, and oligomeric 4R levels in ET cases were not statistically different from PSP, PiD, or AD (Fig. 2F–H). Total 4R to 3R ratios in ET were near 1 regardless of biochemical AT8 levels as is observed in mixed 3R/4R tauopathies (Fig. 2I).

Finally, we assessed differences in N-terminal exons of tau (Fig. 3A–C). Total 0N levels in ET, regardless of AT8 level, were significantly lower than AD, and lower than PSP in the combined ET group (Fig. 3D–F). 0N monomers were significantly lower in the high AT8 and combined ET groups, but the low AT8 ET group was trending down. Conversely, 0N oligomers were significantly lower in the low AT8 and combined ET groups, and the high AT8 ET group was also similarly trending down. Monomeric 1N levels in ET were similar to PSP and PiD but significantly lower than AD when comparing the low AT8 and combined groups (Fig. 3G). Monomeric 2N levels in ET were not significantly different from PSP, PiD, or AD (Fig. 3H).

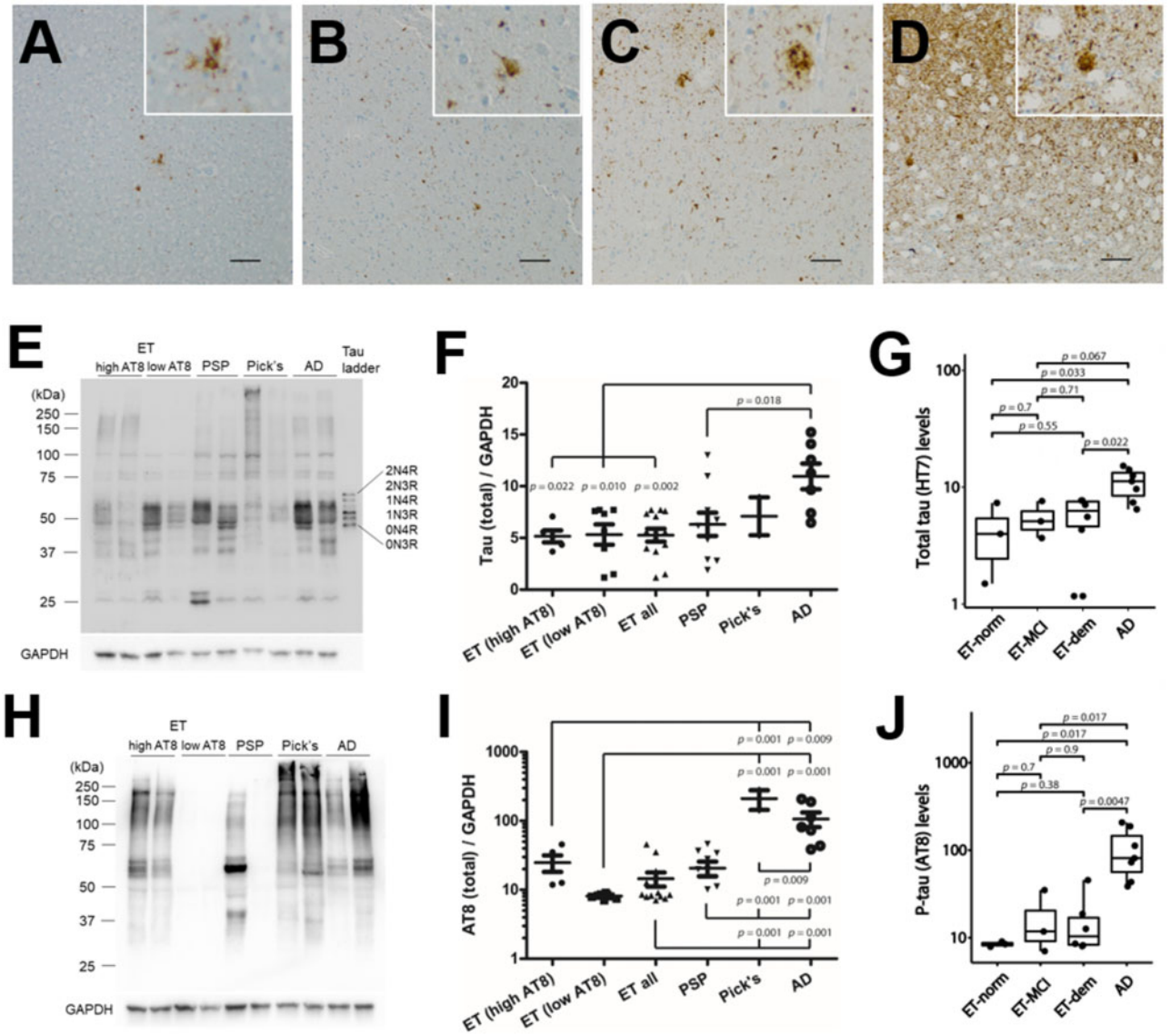

FIGURE 1. Biochemical assessment of tau pathology in essential tremor compared to Alzheimer disease and sporadic tauopathies. (**A–D**) Immunohistochemical studies were performed in parallel samples derived from the fresh frozen temporal cortex tissue block (Brodmann area 37) used for biochemical analysis to assess the presence of tau pathology using antisera recognizing tau phosphorylated at Ser202, Thr205, and Ser208 (p-tau, AT8). This analysis revealed a spectrum from low tau pathology consisting of minimal to scattered thread pathology (**A**, **B**), to high levels of tau pathology, consisting of marked neuronal pathology including neurons and dystrophic neurites (**C**) and/or glial tau pathology (**D**). Immunoblot using antisera targeting total tau (**E–G**) or p-tau (**H–J**) on total protein lysates. ET cases were stratified into high and low p-tau (AT8) groups from biochemical assessments (**F**, **I**) and cognitive status (**G**, **J**). Measurements were normalized to GAPDH. Two data points of PSP cases in (**I**) are outside the axis limits (the values are both 0). Scale bar = 100 μm.

Oligomeric 1N and 2N tau levels were very low or below the threshold of detection and were not quantified.

DISCUSSION

ET is increasingly viewed as a degenerative movement disorder (50–61) that might be variably associated with dementia (7–9). The mechanisms underlying this association are not clear. ET patients may have unique vulnerabilities that differentiate them from other patient populations as ET involves distinct brain systems. Here, we asked whether tau proteostasis in ET differs from other tauopathies by biochemically measuring abnormal cortical p-tau burden and tau isoform ratios compared to other primary and secondary tauopathies. We found that at autopsy, the neuropathological degenerative changes with respect to tau pathology in ET patients are variable and diverge considerably from those with AD. Specifically, the total overall p-tau burden in ET was significantly lower than AD

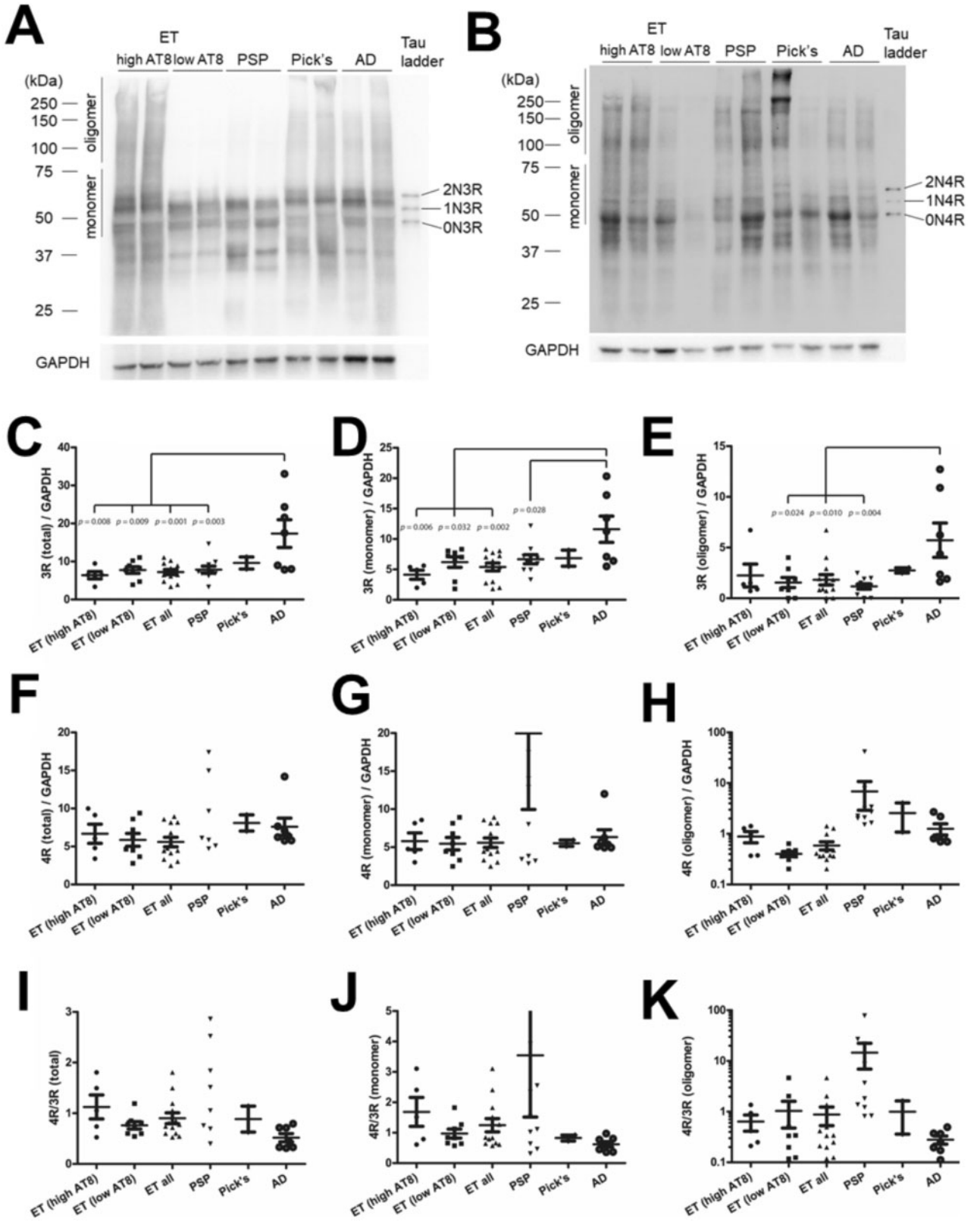

FIGURE 2. Biochemical assessment of 3 and 4 repeat tau isoforms in essential tremor compared to AD and sporadic tauopathies. Representative immunoblots using isoform specific antisera targeting 3R (**A**) or 4R (**B**) on total protein lysates. The relative levels of 3R (**C–E**) and 4R (**F–H**) as well as the ratios of 4R : 3R (**I–K**) normalized to GAPDH are shown. Three data points of PSP cases in (**F**), 2 in (**G**), 2 in (**I**), and 1 in (**J**) are outside the axis limits (the values are 20.6, 34.6, 148.9 in [**F**]; 26.7, 107.1 in [**G**]; 5.0, 21.6 in [**I**]; 21.5 in [**J**]). PSP in (**F**), 26.7 $\pm$ 14.6 (SEM); PSP in (**I**), 3.8 $\pm$ 2.1 (SEM).

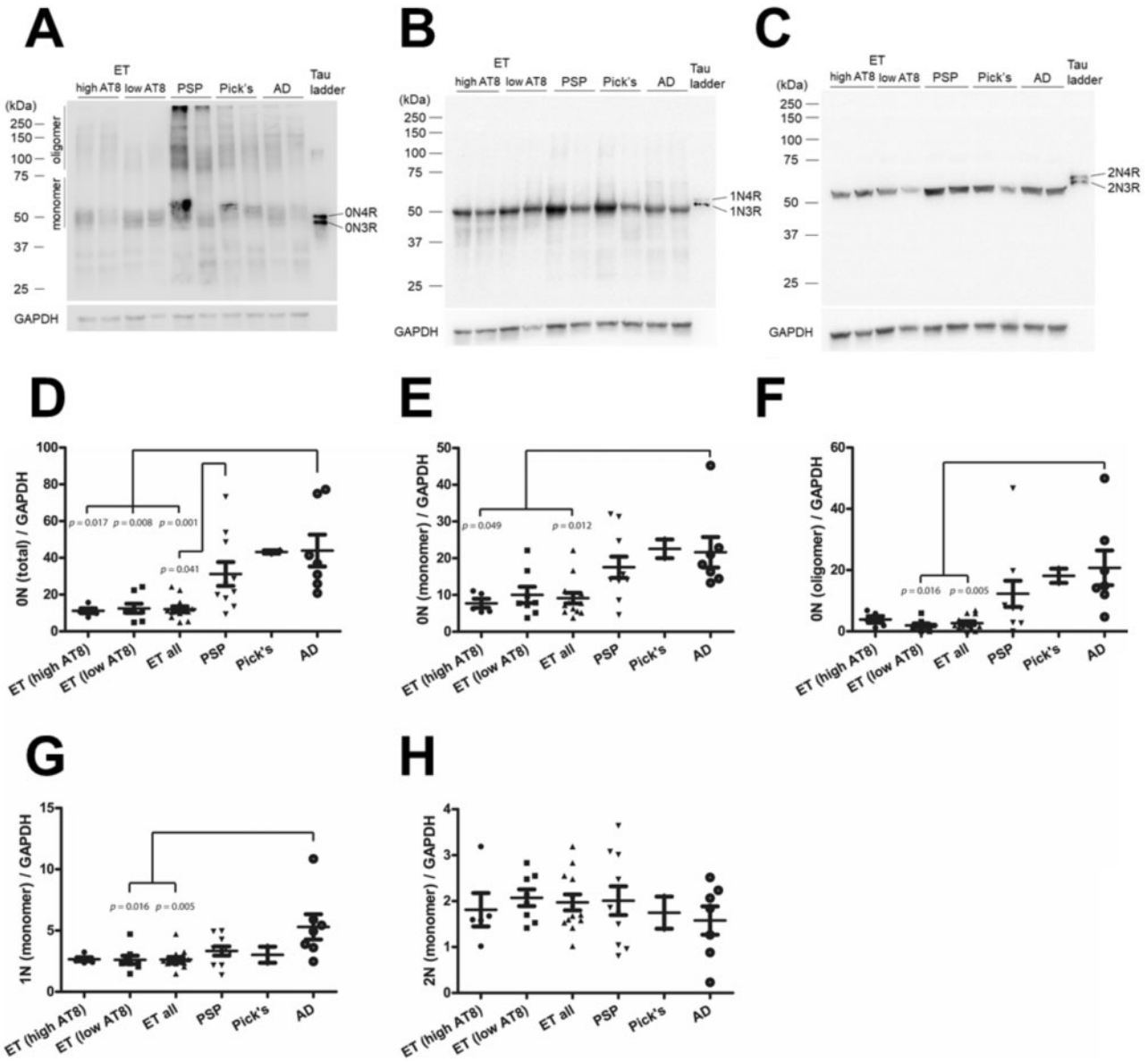

FIGURE 3. Biochemical assessment of N-terminal tau isoforms in essential tremor compared to AD and sporadic tauopathies. Representative immunoblots using isoform-specific antisera targeting 0N (**A**), 1N (**B**), and 2N (**C**) on total protein lysates. The relative levels of tau species are shown normalized to GAPDH (**D**–**H**).

and PiD, albeit these levels were highly variable which is similar to what we observed in PSP. The most striking finding, however, was in the N-terminal isoforms, with markedly lower levels of 0N tau in ET compared to all other groups. Together, these findings provide further evidence that the mechanisms underlying cognitive impairment in ET are divergent from AD and other neurodegenerative disorders.

The ratio of 4R to 3R tau isoforms that result from alternative pre-mRNA splicing of exon 10 are critical diagnostic markers of tauopathy subtypes. One observation was that ET and the primary tauopathies had considerably less 3R tau than AD patients. While AD is generally not thought to shift away from 1 in the tau 4R:3R isoform ratios, this has been previously observed by others (33, 62). We also found that ET and the other disease subtypes did not demonstrate the increase in 4R tau that is observed in PSP. While PSP had a marked elevation of 4R oligomers and monomers, there was a high degree of variability that overlapped with ET.

We further examined differences in tau isoform structure in the N-terminal region, which arises from alternative pre-mRNA splicing of exons 2 and 3. These isoforms, which are of limited use diagnostically, are less well studied. Remarkably, differences in the levels of N-terminal inserts showed the most striking difference. In ET, there were markedly lower levels of 0N tau isoforms compared to both PSP and AD. We also detected significantly lower levels of 1N monomers compared

to AD, but not PSP. The significance of this finding remains unclear. N-terminal exons are outside the MT binding domain and have different binding partners, indicating that it has different function (63–65). The N-terminal region contains intrinsically disordered regions with evolutionary pressure that interact with proteins that play a role in membrane organization (66). The human and murine N-terminus also has important sequence divergence that might underly species-specific differences (67). N-terminal truncation of tau is hypothesized to be a pathogenic event in AD that drives toxicity (68, 69). 0N tau is the fetal isoform and its increase may represent reactivation of developmental pathways that are absent in ET (48). Further efforts to better understand the functional significance of the N-terminal region of tau in neurodegeneration are warranted.

This exploratory study had several notable limitations. First, the samples studied represent a small set of convenience samples from different sources and only include disease-associated tissue. Non-disease associated sample controls are not included, which limits conclusions from this study. It will be critical to conduct a larger study that fully incorporates a broader range of potential covariates. Nevertheless, while we recognize that several of our diagnostic groups differed by age, yet with rare exceptions, age was not associated with tau isoform data, and therefore could not have served as a confounding factor. The immunoblots and immunohistochemistry both rely on specificity of antisera and certain epitopes may be missed due to specific protein conformation and structures that influence antibody binding (70). Also, we focused on the temporal neocortex, an important part of the brain with vulnerability in multiple diseases, but further studies across multiple brain regions are essential to put these findings into the full context or regional vulnerability to tauopathy.

In conclusion, we found that ET patients might exhibit a unique expression signature of tau isoforms that is different from other tauopathies such as PSP, PiD, and AD. Especially, low total 0N tau levels, including oligomers, may be useful for separating ET from the other tauopathies. Levels of AT8-reactive phospho-tau (or corresponding severity of tau tangle pathology), which were not as high as in PiD or AD, does not seem to affect overall tau isoform expression patterns in ET. Additionally, nearly equal levels of 3R and 4R tau isoforms were found in ET, suggesting that ET is more similar to the 3R/4R tauopathies, but there is a high degree of variability that requires further investigation. Previous studies showed that non-demented ET patients had a higher Braak stage and higher number of NFT-positive neurons in the neocortex but not in the medial temporal lobe compared to age- and CERAD score-matched controls, suggesting a predisposition to tau pathology in the ET (15, 17). Taken together, the current findings are further evidence that clinically relevant tau pathology occurs in ET which represents a cerebral tauopathy with an increased susceptibility to cognitive decline.

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