Tau ImmunoPhenotypes in Chronic Traumatic Encephalopathy Recapitulate those of Aging and Alzheimer's Disease

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ABSTRACT

Traumatic brain injury (TBI) is a risk factor for neurodegenerative disease, including chronic traumatic encephalopathy (CTE). Preliminary consensus criteria define the pathognomonic lesion of CTE as patchy tau pathology within neurons and astrocytes at the depths of cortical sulci. However, the specific tau isoform composition and post-translational modifications in CTE remain largely unexplored. Using immunohistochemistry, we performed tau phenotyping of CTE neuropathologies and compared this to a range of tau pathologies, including Alzheimer's disease, primary age-related tauopathy (PART), aging-related tau astrogliopathy (ARTAG) and multiple subtypes of frontotemporal lobar degeneration with tau inclusions (FTLD-Tau). Cases satisfying preliminary consensus diagnostic criteria for CTE neuropathologic change (CTE-NC) were identified (athletes, n=10; long-term survivors of moderate or severe TBI, n=4) from the Glasgow TBI Archive and Penn Neurodegenerative Disease Brain Bank. In addition, material from a range of autopsy-proven aging-associated and primary tauopathies in which there was no known history of exposure to TBI was selected as non-injured controls (n=32). Each case was then stained with a panel of tau antibodies specific for phosphoepitopes (PHF1, CP13, AT100, pS262), microtubule-binding repeat domains (3R, 4R), truncation (Tau-C3) or conformation (GT-7, GT-38) and the extent and distribution of staining assessed. Cell types were confirmed with double immunofluorescent labeling. Results demonstrate that astroglial tau pathology in CTE is comprised of 4R-immunoreactive thorn-shaped astrocytes, echoing the morphology and immunophenotype of astrocytes encountered in ARTAG. In contrast, neurofibrillary tangles of CTE contain both 3R and 4R tau, with post-translational modifications and conformations consistent with Alzheimer's disease and PART. Our observations establish that the astroglial and neurofibrillary tau pathologies of CTE are phenotypically distinct from each other and recapitulate the tau immunophenotypes encountered in aging and Alzheimer's disease. As such, the immunohistochemical distinction of CTE neuropathology from other mixed 3R/4R tauopathies of AD and aging may rest solely on the pattern and distribution of pathology.

KEYWORDS: Chronic traumatic encephalopathy, tau, aging-related tau astrogliopathy, traumatic brain injury, TBI

INTRODUCTION

There is increasing recognition of the association between exposure to traumatic brain injury (TBI) and risk of subsequent neurodegenerative disease, in particular chronic traumatic encephalopathy (CTE). First described as the "punch drunk syndrome" in 1928 (Martland, 1928) and later "dementia pugilistica" (DP) (Millspaugh, 1937), progressive neurodegeneration in the context of TBI was historically considered a consequence of participation in boxing. However, more recent descriptions of the neurodegenerative pathology of DP, now recognized as CTE, in non-boxer athletes exposed to repetitive mild TBI (Corsellis *et al.*, 1973; Geddes *et al.*, 1999; Omalu *et al.*, 2011; Saing *et al.*, 2012; McKee *et al.*, 2013; Smith *et al.*, 2013; McKee *et al.*, 2016; Stewart *et al.*, 2016; Johnson *et al.*, 2017; Mez *et al.*, 2017; Wilson *et al.*, 2017; Lee *et al.*, 2019; Smith *et al.*, 2019) and late survivors of a single moderate or severe TBI (Johnson *et al.*, 2012; Kenney *et al.*, 2018; Zanier *et al.*, 2018) have brought the potential lifelong consequences of TBI exposure to wider attention. Nevertheless, the current consensus criteria for CTE neuropathological assessment remain preliminary and there are few detailed accounts of its similarities and differences with other tauopathies.

While intraneuronal tau aggregates in the form of neurofibrillary tangles (NFTs) have long been described in DP/CTE, more recent studies also note the presence of pathological astroglial tau accumulation in the form of thorn-shaped astrocytes (TSA) (Ikeda *et al.*, 1995; Ikeda *et al.*, 1998; Schmidt *et al.*, 2001; McKee *et al.*, 2009; Saing *et al.*, 2012; McKee *et al.*, 2013; Kanaan *et al.*, 2016; Mez *et al.*, 2017). Indeed, the importance of this mixed neuronal and astroglial pathology in CTE is reflected in preliminary consensus diagnostic criteria for the disease, which propose the pathognomonic lesion as "phosphorylated-tau aggregates in neurons, astrocytes, and cell processes around small vessels in an irregular pattern at the depths of the cortical sulci" (McKee *et al.*, 2016). This sulcal lesion is suggested as sufficiently unique to distinguish CTE from other tau associated neurodegenerative diseases including Alzheimer disease (AD), frontotemporal lobar degeneration characterized by tau inclusions (FTLD-tau) - including progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and Pick's disease (PiD) (Cairns *et al.*, 2007; Montine *et al.*, 2012; Kovacs, 2015), as well as the pathologies of primary age-related tauopathy (PART) and aging-related tau astrogliopathy (ARTAG) (Crary *et al.*, 2014; Kovacs *et al.*, 2016).

However, there remains debate regarding whether tau found at sulcal depths is solely found in individuals with a history of head impacts, or if it can occur in individuals with no contact sport or TBI history (Iverson *et al.*, 2019).

Beyond the potentially unique distribution of tau pathologies in CTE, little is known regarding tau composition and post-translational modifications. Of the six tau isoforms containing either 3 or 4 microtubule-binding repeat domains (3R vs 4R tau) (Goode *et al.*, 2000), NFTs in AD and PART contain both 3R and 4R tau (Espinoza *et al.*, 2008; Santa-Maria *et al.*, 2012; Crary *et al.*, 2014). In contrast, while the Pick bodies and ramified astrocytes of PiD contain primarily 3R tau (Irwin *et al.*, 2016), the astrocytic tau pathologies of PSP, CBD and ARTAG are comprised of 4R tau (Cairns *et al.*, 2007; Ferrer *et al.*, 2014). Regarding CTE pathology, biochemical analysis of material from two former boxers with DP/CTE reported NFTs comprising both 3R and 4R tau, similar to AD (Schmidt *et al.*, 2001; McKee *et al.*, 2013). A further case series indicates that tau in CTE displays conformations and phosphoepitopes comparable to those observed early in NFT maturation in AD (Kanaan *et al.*, 2016). Nonetheless, comprehensive assessment of the phosphorylation, truncation and conformation of both glial and neuronal tau pathologies in CTE has not previously been assessed, particularly within the context of other established tauopathies (Ferrer *et al.*, 2014; Kovacs, 2015; Irwin *et al.*, 2016).

Here, we report immunohistochemical observations on extensive tau phenotypic analysis of autopsy-derived material from individuals with known CTE neuropathologies and histories of exposure to either repetitive sport-related mild TBI, or moderate/severe TBI, when compared to aging-associated and primary neurodegenerative tauopathies. Specifically, applying antibodies specific for tau isoform, multiple phosphoepitopes, caspase-mediated truncation, and novel monoclonal antibodies capable of differentiating 3R/4R tau in AD from the conformationally-distinct FTLD-tau (Gibbons *et al.*, 2018; Gibbons *et al.*, 2019), we demonstrate that while the NFTs and astroglial tau pathologies of CTE differ immunohistochemically from each other, they recapitulate features of AD/PART and ARTAG, respectively.

MATERIALS AND METHODS

Cohort Demographics

All cases were obtained from the Glasgow TBI Archive, Department of Neuropathology, Queen Elizabeth University Hospital, Glasgow, UK or the University of Pennsylvania Center for Neurodegenerative Disease Research (CNDR) Brain Bank, Philadelphia, PA, USA. Brain tissue was acquired by means of planned donation or at routine diagnostic autopsy. Ethical approval for use of tissue in this study was provided by the West of Scotland Research Ethics Committee (Project ID 225271); and the Greater Glasgow and Clyde Biorepository (Application Number 340), as well as the institutional review board of the University of Pennsylvania.

Cases were selected with a history of participation in contact sports: American football (n=4), rugby (n=3), soccer (n=2), or boxing (n=1), or a remote history of single moderate or severe TBI caused by assault (n=1), motor vehicle collision (n=1) or fall (n=1). One additional case sustained one mild and one moderate TBI caused by falls (n=1). Cases were selected with previously confirmed CTE neuropathologic change (CTE-NC) (Lee *et al.*, 2019) based on the preliminary consensus criteria for the neuropathological evaluation of CTE (McKee *et al.*, 2016). Notably, in addition to CTE neuropathology, multiple cases also displayed co-morbid neuropathologies (n=8) as has previously been reported (Lee *et al.*, 2019). Clinical, demographic and neuropathologic information, including integrated clinicopathologic diagnoses (Lee, 2018) for all cases is presented in **Table 1**.

To permit comparisons of CTE tau pathologies with those of established neurodegenerative disease, material from patients without documented history of TBI or participation in contact sport was selected that met neuropathological criteria for the diagnosis of AD (Braak Stage V or VI; n=6) or FTLD-tau as Pick's disease (PiD) (n=6), PSP (n=6), or CBD (n=6). In addition, non-demented controls without history of TBI and with known aging-related tau pathologies were selected including; PART (n=1), ARTAG and PART (n=3) and ARTAG with low AD neuropathologic change (n=4) (Cairns *et al.*, 2007; Montine *et al.*, 2012; Crary *et al.*, 2014; Kovacs *et al.*, 2016; McKee *et al.*, 2016) (**Table 1**).

Brain Tissue Handling

Whole brains from the Glasgow TBI Archive were fixed in 10% formol saline at autopsy for a minimum of two weeks prior to dissection. Standardized anatomical sampling, tissue processing and paraffin embedding were performed as previously described (Graham *et al.*, 1995). From the University of Pennsylvania CNDR Brain Bank, tissue blocks cut from fresh brains were fixed overnight in 70% ethanol and 150mMol sodium chloride or 10% neutral buffered formalin and processed to paraffin as previously described (Toledo *et al.*, 2014). From each case, 8μm tissue sections were prepared from regions with stereotypical CTE neuropathology at the depths of cortical sulci. For comparison, regions displaying hallmark, disease-specific pathologies were selected from non-trauma control cases of AD (NFTs), PART (NFTs), PiD (Pick bodies and ramified astrocytes), PSP (tufted astrocytes), CBD (astrocytic plaques) and ARTAG (TSA) (Cairns *et al.*, 2007; Montine *et al.*, 2012; Crary *et al.*, 2014; Kovacs *et al.*, 2016).

Single Immunohistochemical Labeling

Serial tissue sections for all cases were subjected to deparaffinization and rehydration to H_2O before being immersed in 3% aqueous H_2O_2 (15 minutes) to quench endogenous peroxidase activity. Antigen retrieval was performed via microwave pressure cooker in either Tris/EDTA or citrate buffer, with or without formic acid pre-treatment, as optimized for each antibody (**Table 2**). Sections were blocked using normal horse serum (Vector Labs) in Optimax buffer (BioGenex) for 30 minutes followed by incubation in the primary antibody overnight at 4°C. Specifically, a panel of tau antibodies (**Table 2**) was applied targeting multiple phosphoepitopes including S202 (CP13) (Jicha *et al.*, 1999), S396/S404 (PHF1) (Greenberg *et al.*, 1992; Otvos *et al.*, 1994), S212/T214 (AT100) (Hoffmann *et al.*, 1997; Zheng-Fischhofer *et al.*, 1998) and S262; 3 or 4 microtubule-binding domain repeats (RD3 & RD4) (de Silva *et al.*, 2003); caspase-cleaved tau at Asp421 (Tau-C3) (Gamblin *et al.*, 2003). In addition we applied the recently characterized antibodies GT-7 & GT-38 (Gibbons *et al.*, 2018; Gibbons *et al.*, 2019). Evidence from co-immunofluorescence studies in human tissue with FTLD-tau and AD-tau suggest that GT-38 requires the presence of both 3R and 4R tau. Moreover, it was demonstrated that GT-38 binding requires a pathological conformation of AD-tau since chemical denaturation leads to a reduction of GT-38 binding (Gibbons, G. S. et al. 2018 J Neuropathol Exp Neurol 77, 216-228).

After rinsing, sections were incubated in a biotinylated universal secondary antibody (Vector Labs) for 30 minutes, followed by the avidin-biotin complex for 30 minutes (Vector Labs).

Visualization was achieved using the DAB peroxidase substrate kit (Vector Labs). Sections were counterstained with hematoxylin, followed by rinsing, dehydration, and coverslipping using cytoseal 60. Tissue sections from a case with neuropathologically confirmed AD were included as a positive control in all staining procedures. Omission of the primary antibody using the same AD case was performed in parallel to control for non-specific binding. Notably, 3 cases (**Table 3**: Cases 9 (CTE neuropathology), 11 (CTE neuropathology) and 15 (ARTAG/PART)) failed to demonstrate immunoreactivity to antibodies specific for 3R and 4R tau due to fixation sensitivity, as has been reported previously with these antibodies (Espinoza *et al.*, 2008; Ferrer *et al.*, 2014).

Double Immunofluorescent Labeling

Serial tissue sections from a subset of cases (CTE n=5, ARTAG n=2, AD n=2) were selected for double labeling immunofluorescence to confirm and validate morphological identification of cell types (astrocytes versus neurons) as identified by both PHF1 and GT-38. Specifically, sections were labelled with combinations of tau antibodies (PHF1 or GT-38) and cell-type specific markers, namely MAP2 for neurons and GFAP for astrocytes using established protocols (Johnson *et al.*, 2016). Briefly, following deparaffinization and rehydration, antigen retrieval was performed as described above and tissue blocked in the relevant species-specific serum (1%) (Vector Labs). Primary antibodies were applied serially for 20 hours (4^{o}C) specific for PHF1 (1:100) or GT-38 (1:100), followed by glial fibrillary acidic protein (GFAP) (Abcam, Cambridge, MA; 1:200) or the microtubule-associated protein 2 (MAP2) (Abcam, Cambridge, MA; 1:200). After rising, the corresponding Alexa Fluor (Invitrogen, Carlsbad, CA) secondary antibody was applied at 1:500 in a 2% species-specific blocking solution for 2 hours at room temperature. Serial sections of positive control tissue (AD) were subjected to the entire procedure with omission of subsets of primary antibodies to control for non-specific immunofluorescence. Following rinsing, all double fluorescent-immunolabeled sections were incubated in TrueView autofluorescence quenching reagent (Vector Labs) for 5 minutes at room temperature before being rinsed and coverslipped using Vectashield mounting medium (Vector Labs).

Analysis of Immunohistochemical Findings

Using a standardized approach, the extent of immunoreactivity for each antibody was scored relative to that observed using an index antibody in each individual case, as has been described previously (Ferrer *et al.*, 2014). Specifically, a semi-quantitative score was used to

denote the extent of immunoreactivity relative to that of PHF1 as: absent or nearly absent (<5% concordance with the extent of PHF1 immunoreactivity): minimal (5-30% concordance); moderate (30-70% concordance); or extensive (>70% concordance). PHF1 was selected as the index antibody given its widely reported use for the identification of tau pathologies across neurodegenerative diseases and recommended use for the identification of CTE pathology in preliminary consensus criteria (McKee *et al.*, 2016). Under this protocol, scoring does not reflect the number of positive cells or permit comparisons of the extent of pathology between cases, but rather reflects the relative extent to which a particular antibody recognizes the burden of tau pathology as identified via PHF1 in each field of interest. Glial versus neuronal pathologies were distinguished based on characteristic cellular morphologies. A subset of sections was reviewed and scored independently by two observers (JDA & VEJ), with good interrater reliability (Cohen's Kappa 0.71). Where there was a discrepancy in scoring, cases were jointly reviewed and a consensus score reached.

RESULTS

Consistent with prior descriptions of CTE neuropathology (McKee *et al.*, 2016), each TBI case displayed PHF1 positive neurons and astrocytes in a patchy and perivascular distribution concentrated at the depths of cortical sulci **(Fig 1)**. Astrocytes typically displayed thorn-shaped morphologies, with short, thickened processes (Ikeda *et al.*, 1995; Kovacs *et al.*, 2016; Kovacs *et al.*, 2017a), frequently in the immediate subpial region at the sulcal depth, in addition to being observed in a patchy and perivascular distribution within deeper layers of cortex. Neuronal tau pathology at the depths of cortical sulci in cases with CTE neuropathology displayed the morphology of NFTs, consistent with historical and contemporary descriptions (Corsellis *et al.*, 1973; Geddes *et al.*, 1996; Geddes *et al.*, 1999; Omalu *et al.*, 2005; McKee *et al.*, 2009; McKee *et al.*, 2013; McKee *et al.*, 2016). The distinctive cellular morphologies of CTE astrocytes and neurons were confirmed via double immunofluorescence labeling on a subset of cases as described. Specifically, NFTs identified via PHF1 co-localized with MAP2, but not GFAP. Conversely, thorn-shaped astrocytes (TSA) were observed to co-localize with GFAP but not MAP2, consistent with findings in AD and ARTAG **(Figs 7, S1, S2)**.

As expected, neurodegenerative disease controls displayed the hallmark and cell-specific tau pathologies characteristic for each diagnosis as identified by PHF1. Specifically, FTLD-tau

controls demonstrated Pick bodies and, in some cases, ramified astrocytes in PiD, tufted astrocytes in PSP and astrocytic plaques in CBD. AD cases had NFTs in a bilaminar cortical distribution in addition to diffuse neuritic threads. Controls with aging related pathologies displayed limited cortical NFT pathology consistent with PART or low AD neuropathological change, as well as TSA consistent with ARTAG in white matter, perivascular, gray matter, subpial, or subependymal distributions.

Astroglial pathology of CTE contains 4R tau only, whereas neurofibrillary tangles are comprised of both 3R and 4R tau

Tau positive astrocytes within regions of CTE neuropathology were composed of 4R tau only in virtually all cases **(Figs 2 & 3; Table 3)**. Specifically, in cases demonstrating adequate immunoreactivity, robust and consistent immunoreactivity to RD4 was observed in astrocytes within the subpial region, as well as those extending to deeper cortical layers. In contrast, astrocytic immunoreactivity to the RD3 antibody was absent in all but two cases where just minimal staining was observed. Notably, TSA of CTE were morphologically indistinguishable from those within non-injured control cases with ARTAG, which were also comprised almost entirely of 4R tau, consistent with previous reports (Lopez-Gonzalez *et al.*, 2013; Ferrer *et al.*, 2018) **(Fig 3)**. Several cases with CTE neuropathology displayed TSA elsewhere in the tissue sections examined, including within subcortical white matter, subependymal, and subpial regions, in keeping with descriptions of ARTAG. These astrocytes were morphologically indistinguishable from those in the sulcal depths associated with stereotypical CTE neuropathology and displayed the same pattern of selective 4R tau immunoreactivity.

In contrast, ramified astrocytes of Pick's disease were comprised of 3R tau, with a subset of cells in just one of the six cases also displaying 4R immunoreactivity, as has been reported previously in a subset of cases (Arai *et al.*, 2001; Ferrer *et al.*, 2014; Irwin *et al.*, 2016). The tufted astrocytes in PSP and astrocytic plaques in CBD controls displayed immunoreactivity for 4R tau only **(Fig 3)**.

While astrocytes in CTE were typically 4R tau-immunoreactive / 3R tau-negative, NFTs in these regions were immunoreactive for both 3R and 4R tau isoforms, similar to those of AD and PART **(Fig 2; Table 3)** and distinct from Pick bodies (3R positive only) **(Fig 2)**.

Post-translational modification of tau in CTE neuropathology is consistent with ARTAG and AD

The post-translational modifications of tau within astrocytes and NFTs of CTE were observed to recapitulate those of ARTAG and AD with respect to all antibodies assessed. Specifically, tau immunoreactive astrocytes in CTE exhibited phosphorylation at residues S202 (CP13), S212/T214 (AT100), S262, and S396/S404 (PHF1) **(Fig 4, Table 4)**. Typically, CP13, AT100, and PHF1 displayed dense cytoplasmic staining throughout the cell body. In contrast, pS262 immunoreactivity displayed both robust and confluent immunoreactivity **(Fig 4)**, as well as a more dot-like pattern of immunoreactivity in a subset of cases/cells that was often concentrated in the peripheral processes of the astrocyte **(Fig S3)**. Caspase-mediated truncation at D421 (Tau-C3), however, was virtually never seen in astrocytes in CTE, with just minimal cells observed in a single case. Again, all findings were consistent across TSA in subpial and deeper cortical astrocytes within the sulcal depth. This profile of staining was also indistinguishable from that observed in non-injured, ARTAG control cases **(Fig 4)**. In contrast with the astroglial pathologies of CTE, ARTAG, PiD and CBD, which did not typically demonstrate immunoreactivity to Tau-C3, subsets of tufted astrocytes in two PSP cases demonstrated Tau-C3 immunoreactivity, consistent with previous reports **(Fig 4)** (Ferrer *et al.*, 2014).

Notably, NFTs in CTE also displayed robust cytoplasmic immunoreactivity for all tau phosphoepitopes including S202 (CP13), S212/T214 (AT100), S262 and S396/S404 (PHF1) **(Fig 5)**. Moreover, a sub-population of NFTs in CTE demonstrated evidence of caspase-mediated truncation of tau at D421 (Tau-C3) **(Fig 5)**, consistent with that observed previously (Kanaan *et al.*, 2016), and in AD here and in prior reports (Gamblin *et al.*, 2003).

FTLD-tau controls were consistent with prior characterizations performed using these antibodies (Buee and Delacourte, 1999; Guillozet-Bongaarts *et al.*, 2007; Ferrer *et al.*, 2014), and displayed immunoreactivity for antibodies recognizing phosphorylation at residues S202 (CP13), S212/T214 (AT100), S262 (p262), and S396/S404 (PHF1). Notably, in one case of PiD, Picks bodies appeared weakly immunoreactive for p262 **(Fig 5)**. In contrast, neurons were otherwise negative for tau p262 in all other five PiD cases. Notably, previous work has demonstrated conflicting results with regard to pS262 immunoreactivity in PiD (Probst *et al.*, 1996; Delacourte *et al.*, 1998; Ferrer *et al.*, 2002; Zhukareva *et al.*, 2002; Irwin *et al.*, 2016; Falcon *et al.*, 2018). However, greater immunoreactivity has been reported in ethanol versus

formalin fixed tissue (Irwin *et al.*, 2016), consistent with our observations. Moreover, the intensity of pS262 immunoreactivity was reported as greater in cases with 4R tau inclusions (Zhukareva *et al.*, 2002).

Neurofibrillary tangles, but not astrocytes, in CTE show a similar conformational profile to those in AD

Recently developed conformation-selective antibodies, GT-7 and GT-38, have been shown to detect a conformation dependent epitope present in tau within the inclusions of AD requiring both 3R and 4R tau, but not the 3R or 4R tau-only inclusions of other primary tauopathies, with both antibodies labeling AD-tau in a phosphorylation-independent manner (Gibbons *et al.*, 2018; Gibbons *et al.*, 2019). In CTE neuropathology, both GT-7 and GT-38 showed moderate to strong labelling of NFTs at the depths of sulci, consistent with AD **(Fig 6, Table 3)**. However, in contrast with NFTs, astrocytes within CTE were negative for GT-7 or GT-38 in all but four cases in which there was very occasional and minimal positivity to one or the other antibody **(Table 3)**. These cells did not differ in their morphology, and no notable differences in fixation, clinical history or anatomic distribution distinguished them from the rest of the cohort. Double immunofluorescence labeling in a subset of cases confirmed neuron-specific colocalization of GT-38 with MAP-2, and an absence of co-localization with GFAP **(Fig 7, S2)**.

As anticipated, NFTs in non-injured AD and PART cases demonstrated robust immunoreactivity for both antibodies **(Fig 6, Table 3)**, while Pick bodies and the glial profiles of ARTAG, CBD and PSP were negative for both GT-7 and GT-38 **(Fig 6).** Notably, the ramified astrocytes within the single PiD case that were immunoreactive for both 3R and 4R tau, were not immunoreactive for GT-7 or GT-38. Among TBI cases with co-morbid diagnoses of FTLD-tau, both GT-7 and GT-38 antibodies stained the characteristic NFTs of CTE but not the adjacent FTLD-tau disease-associated pathologies within the same tissue section.

DISCUSSION

Here, we performed immunohistochemical characterization of tau phenotypes within the cellular constituents of CTE in patients with known exposure to repetitive mild TBI or moderate/severe TBI. Intriguingly, it was found that the tau species within NFTs and immediately adjacent astrocytes in CTE are phenotypically distinct. Specifically, the NFTs of

CTE displayed an immunophenotype that mirrored that seen in AD or PART, including being both 3R and 4R immunoreactive and positive for antibodies previously shown to bind to a conformation dependent epitope present within the tau inclusions of AD. In contrast, the astroglial component of CTE was solely 4R immunoreactive and without evidence of AD conformation, which was indistinguishable from ARTAG. Notably, while the neuronal and glial pathologies of CTE showed similarity with those of AD, PART and ARTAG, they were distinct from those of the primary FTLD tauopathies of PiD, CBD and PSP. As such, our data suggest that while pattern and distribution of involvement might be distinct, the tau pathologies of CTE show considerable overlap with the immunoreactivity profiles of both aging-related tau pathologies and AD.

Typically, we observed tau immunoreactive astrocytes within CTE to have the characteristic morphology of TSA, in keeping with those encountered in ARTAG, in both the non-injured control material studied here and in multiple other reports (Lopez-Gonzalez *et al.*, 2013; Kovacs *et al.*, 2016; Kovacs *et al.*, 2017a; Kovacs *et al.*, 2018a). Notably, TSA have long been described in material from patients with and without concomitant neurodegenerative disease. In particular, their appearance in association with increased age led to the recognition of the specific entity recently defined as ARTAG, wherein TSA are described in subpial, subependymal and perivascular distributions (Ikeda *et al.*, 1995; Ikeda, 1996; Ikeda *et al.*, 1998; Schultz *et al.*, 2004; Lace *et al.*, 2012; Kovacs *et al.*, 2013; Kovacs *et al.*, 2016). While the clinical significance of ARTAG has yet to be fully explored (Kovacs *et al.*, 2017b), the morphological resemblance between astrocytes immunoreactive for phosphorylated tau in both CTE and ARTAG raises the possibility that these entities share common pathogenic mechanisms (Kovacs *et al.*, 2016; Liu *et al.*, 2016; Kovacs *et al.*, 2017b; Goldfinger *et al.*, 2018; Kovacs *et al.*, 2018a; Forrest *et al.*, 2019). Our data demonstrate that the astrocytes of CTE and ARTAG not only share similar morphologies, but display indistinguishable tau immunophenotypes with respect to the panel of antibodies applied. Specifically, consistent with previous reports characterizing ARTAG (Kovacs *et al.*, 2016; Ferrer *et al.*, 2018) and one limited description in CTE (McKee *et al.*, 2013), TSAs in CTE were typically 4R tau-immunoreactive/3R tau-negative, with only very occasional cells displaying immunoreactivity for 3R tau in a subset of cases. Further, astrocytes in CTE demonstrated a profile of immunoreactivity for multiple phospho-epitopes of tau consistent with descriptions of ARTAG here and previously (Lopez-Gonzalez *et al.*, 2013; Ferrer *et al.*, 2018).

Beyond this astroglial pathology, the regionally co-existing NFTs in CTE appeared morphologically and phenotypically consistent with those of AD and PART, comprised of both 3R and 4R tau, and immunoreactive for tau hyperphosphorylated at multiple phospho-epitopes, including S202, S212/T214, S262, and S396/S404. Notably, characterization by others has highlighted additional shared features of tau including phosphatase-activating domain exposed conformation (TNT1 antibody), tau oligomers (TOC1), and truncation at D421 (Tau-C3) (Kanaan *et al.*, 2016). Here we demonstrate that a subset of NFTs in AD, PART and CTE demonstrate caspase-mediated truncation at D421. Interestingly, Tau-C3 positivity has previously been reported as relatively sparse in CTE neuropathology when compared with AD (Kanaan *et al.*, 2016). However, the diminished total burden of Tau-C3 immunoreactivity may reflect a virtual absence of immunoreactivity in TSA, as observed here. Truncation of tau at D421 has been reported as an early event in the evolution of NFTs in AD (Gamblin *et al.*, 2003; Rissman *et al.*, 2004; Cotman *et al.*, 2005), although it may not be essential for filament formation (Delobel *et al.*, 2008). Moreover, experimental data indicates caspase-mediated truncation may promote polymerization and seeding of full length tau (Abraha *et al.*, 2000; Berry *et al.*, 2003; Gamblin *et al.*, 2003; Rissman *et al.*, 2004), as well as contribute to neurotoxicity via apoptosis (Fasulo *et al.*, 2000; Chung *et al.*, 2001; Fasulo *et al.*, 2005). As such, the relative absence of Tau-C3 immunoreactivity in TSA of CTE may have implications as to the potential pathological nature of tau immunoreactive TSA in CTE.

The astrocytic tau pathologies of both CTE and ARTAG also failed to display immunoreactivity for the recently developed tau antibodies, GT-7 and GT-38, previously demonstrated to detect a conformation-dependent epitope of tau in AD in a phosphorylation-independent manner (Gibbons *et al.*, 2018; Gibbons *et al.*, 2019). Furthermore, these antibodies failed to label pathologies comprised of tau with either 3R or 4R isoforms only (Gibbons *et al.*, 2018; Gibbons *et al.*, 2019). Thus, the observation that both GT-7 and GT-38 bind to NFTs in CTE, but not sulcal TSA, further supports the observation that astrocytic tau is 4R only and differs from that of the adjacent NFTs. Indeed, while the characteristic pathologies of FTLD-tau were not immunoreactive for either GT-7 or GT-38, as previously characterized in detail (Gibbons *et al.*, 2018; Gibbons *et al.*, 2019), the NFTs of CTE, AD and PART were consistently immunoreactive for both.

The panel of antibodies examined herein were selected for their previous extensive characterization and reported differences across a range of neurodegenerative diseases. As no immunolabel examined thus far can morphologically or phenotypically differentiate individual cells in CTE from those of other established tauopathies, the distinguishing features of this pathology remain dependent on the overall pattern and distribution of pathology when using immunohistochemistry (McKee *et al.*, 2016). Notably, this panel is not exhaustive, and the use of immunohistochemistry to explore additional, and potentially distinguishing tau phenotypes, including ubiquitination and acetylation, will be important. Notably, a recent study based on cryo-electron microscopy (cryo-EM) analysis of temporal lobe tissue from 3 cases of known CTE suggests that the tau filament structure of CTE is distinct from that of AD (Falcon *et al.*, 2019). However, it is noteworthy that all three cases demonstrated clinical or neuropathological evidence of other neurodegenerative disease, including Parkinson's disease, FTLD and/or motor neuron disease, in one instance associated with C9orf72 mutation (Falcon *et al.*, 2019). Given the diversity and heterogeneity of co-morbid pathologies reported with CTE here and elsewhere (Mez *et al.*, 2017; Lee *et al.*, 2019), it will be important to extend cryo-EM studies to a wider range of trauma-associated cases. Nonetheless, these data suggest that while tau in CTE might differ from that of other established neurodegenerative disease, in particular AD, this may only be detectable by means beyond established immunohistochemical or biochemical approaches.

There is increasing recognition that mixed, often multiple pathologies might co-exist in patients with neurodegenerative disease, including in those with TBI-related neurodegeneration where CTE might serve as the primary pathology driving disease or as a co-morbidity in context of an alternate diagnosis (Lee *et al.*, 2019). Consistent with this, most cases with CTE examined here also met criteria for other tauopathies, including AD, PSP, and CBD, as previously reported (Lee *et al.*, 2019). Notably, the morphologies and immunophenotype of CTE were highly consistent across cases, regardless of the presence or extent of co-morbid disease. Moreover, CTE was consistent in phenotype, regardless of the nature of TBI exposure, including in three cases with a remote history of single moderate or severe TBI. These cases add to the limited number of described cases of single TBI associated with CTE neuropathologies, supporting the assertion that it is exposure to TBI rather than severity or number of injuries that serves as the primary risk factor for CTE (Smith *et al.*, 2013; Maroon *et al.*, 2015; Smith *et al.*, 2019).

Collectively, these data indicate the co-existence of distinct tau phenotypes within neurons and astrocytes contributing to CTE neuropathology. Moreover, immunohistochemical observations were notably consistent across all cases examined, comprised of diverse TBI exposure histories. While criteria for delineating the extent or stage of disease in CTE have yet to be adequately defined, future explorations of tau immunophenotype in association with disease progression will be of importance to examine.

While the mechanisms driving tau pathology following TBI exposure remain poorly understood, it is possible that the differential neuronal and astrocytic components reflect mechanistically independent pathological processes. Curiously, TSA in both ARTAG and CTE are frequently observed at brain parenchyma-fluid interfaces, including subpial, subependymal and perivascular regions (Ikeda *et al.*, 1995; Geddes *et al.*, 1996; Geddes *et al.*, 1999; Kovacs *et al.*, 2016; McKee *et al.*, 2016; Kovacs *et al.*, 2018a). Moreover, regional correlation of ARTAG with astrocytic expression of connexin-43 and aquaporin 4 has led to speculation that blood brain barrier (BBB) dysfunction may be of pathologic significance to the development of this pathology (Kovacs *et al.*, 2018b). While BBB dysfunction is increasingly recognized as an important acute pathology of TBI, even following concussion (Weissberg *et al.*, 2014; Johnson *et al.*, 2018), recent data indicates BBB permeability may persist chronically in some individuals after severe TBI (Hay *et al.*, 2015) and has also been described in cases of CTE (Doherty *et al.*, 2016). However, a potential mechanistic relationship between BBB dysfunction and pathological astrocytic tau accumulation remains unexplored.

Here we provide new insights into the nature of tau in CTE neuropathologic change directly within the context of other neurodegenerative pathologies. Moreover, these data highlight the potential challenges in distinguishing trauma-associated tau pathologies from those of other diseases at the individual cell level using immunohistochemistry alone. Nonetheless, morphological and phenotypic similarities between tau in CTE and those of ARTAG suggests the intriguing possibility of shared pathogenic mechanisms.

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FIGURE LEGEND

Figure 1. Sulcal depth astrocytic and neuronal tau pathologies in CTE. (a,b) PHF1 immunohistochemistry reveals neurofibrillary tangle and thorn-shaped astrocyte pathology concentrated at the depths of cortical sulci of a former American football player (Case 3) and **(c,d)** chronic survivor of a single severe TBI (Case 11). **(b,d)** High magnification images from the same sulci showing perivascular pathology comprised of mixed neuronal and astrocytic populations consistent with the preliminary diagnostic criteria for CTE. *All scale bars 100 μm.*

Figure 2. 3R versus 4R tau immunoreactivity in CTE, AD, PART and PiD. (a) Sulcal depth CTE neuropathology with prominent subpial thorn-shaped astrocytes in addition to patchy and perivascular thorn-shaped astrocytes and neurofibrillary tangles within the deeper layers of cortex (Case 10; PHF1 staining). **(b)** Higher magnification of box in **(a)** displaying perivascular astrocytic and neuronal tau pathologies. **(c)** Immunoreactivity specific for 3R tau in the same region as **(b)** showing perivascular neurofibrillary tangles, but an absence of immunoreactivity within astrocytes. **(d)** In contrast, immunohistochemistry specific for 4R tau identified cells with both neuronal and astrocytic morphologies in the same region. **(e)** Consistent with previous descriptions, cases meeting diagnostic criteria for AD (Case 25) and PART (Case 15) displayed neurofibrillary tangles in the cortex that were immunoreactive for 3R and 4R tau. In contrast, Pick bodies of PiD within the dentate granule cells of Case 29 were composed of only 3R tau. *Scale bars **a-d** 100 μm, **e** 50 μm.*

Figure 3. 3R versus 4R tau immunoreactivity within the astrocytic pathologies of CTE, ARTAG, PSP, CBD and PiD. Representative examples of serial sections showing

immunoreactivity specific for 4R tau, but not 3R tau, in the thorn-shaped astrocytes in cases of both CTE (Case 6) and ARTAG (Case 21), tufted astrocytes in a case with PSP (Case 35) and astrocytic plaques within a case of CBD (Case 41). The ramified astrocytes observed in just one case of PiD (case 29) were immunoreactive for 3R and 4R tau. *Scale bars 50 µm.*

Figure 4. Post-translational modifications of tau within astrocytes in CTE, AD, PART, ARTAG, PSP, CBD and PiD. Representative examples of serial sections showing the thorn-shaped astrocytes of CTE (Case 3) and ARTAG (Case 22), astrocytic plaques of CBD (Case 41) and ramified astrocytes in a case of PiD (Case 29) all displayed robust immunoreactivity to phosphoepitope antibodies PHF1, CP13, AT100 and pS262, but not Tau-C3 (truncation at D421). In contrast with all other astrocytic tau pathologies examined, the tufted astrocytes of PSP in a subset of cases (Case 35 pictured here) were labeled with Tau-C3, indicative of truncation as described. *Scale bars 50 µm.*

Figure 5. Post-translational modifications of tau within neurons in CTE, AD, PART and PiD. Representative examples of serial sections showing the neurofibrillary tangles of CTE (Case 5), AD (Case 28) and PART (Case 15) with immunoreactivity to the phosphoepitope antibodies PHF1, CP13, AT100 and pS262, as well as Tau-C3 indicating truncation at D421. In addition, while Pick bodies (Case 29) also demonstrated the same post-translational modifications, immunoreactivity to p262 and Tau-C3 was noted in just one case as shown here within the dentate granule cells, where immunoreactivity was notably less robust. *Scale bars 50 µm*

Figure 6. Neurofibrillary tangles, but not astrocytes, are immunoreactive for antibodies that detect a conformation-dependent epitope of tau in AD. PHF1 immunohistochemistry revealed sulcal depth astrocytic and neuronal tau pathology in CTE (Case 8), including prominent clusters of perivascular and subpial thorn-shaped astrocytes (top left; black arrows). However, GT-7 and GT-38 antibodies labeled neurofibrillary tangles in CTE, but not thorn-shaped astrocytes, on serial tissue sections (top middle and top right). GT-7 and GT-38 reliably detected neurofibrillary tangles in AD (Case 28), but failed to label the characteristic pathologies of ARTAG (Case 19), PiD (Case 29), PSP (Case 35) or CBD (Case 41).
Scale bars: CTE (low power) 100 µm; CTE (high power), AD, PiD, ARTAG, PSP and CBD 50 µm.

Figure 7. Double label immunofluorescence confirms cell-type specific patterns of immunoreactivity for GT-38 in CTE. (a-c) PHF1 and GFAP immunoreactivity showing a cluster of thorn-shaped astrocytes (arrow heads) that co-localize with GFAP. Adjacent neurons (arrows) do not co-localize with GFAP. **(d-f)** From the same region, a serial tissue section shows PHF1 immunoreactive neurons (arrows) that co-localize with MAP2 and nearby thorn-shaped astrocyte (arrow head) lacking MAP2 immunoreactivity. **(g-l)** While GT-38 positive cells fail to co-localize with GFAP, they demonstrate co-localization with MAP2, confirming their neuronal identity. *Scale bars 50 µm.*

Figure S1. Double label immunofluorescence confirms perivascular astrocytes in a case of CTE. (a-c) PHF1 and GFAP immunoreactivity showing a cluster of thorn-shaped astrocytes that co-localize with GFAP positive cells. Cells with the morphological appearance of neurons fail to display any co-localization with GFAP immunoreactivity. *Scale bars 100 µm.*

Figure S2. Double label immunofluorescence labelling in cases of ARTAG and AD. A case with ARTAG and low ADNC displaying **(a-c)** PHF1 immunoreactivity showing a cluster of TSA that fail to co-localize with MAP2. Note the single MAP2 positive / PHF1 negative neuron within in the same field. **(d-f)** TSAs which are PHF1 immunoreactive and that co-localize with GFAP. **(g-i)** GFAP immunoreactive astrocytes in a region observed to have extensive PHF1 positive TSA on serial section, but without immunoreactivity to GT-38. Tissue from AD cases showing **(j-l)** PHF1 immunoreactive NFTs that do not co-localize with GFAP. **(m-o)** PHF1 positive neuron that co-localizes with MAP2. **(p-r)** GT-38 positive neuron that fails to co-localize with GFAP and, **(s-u)** a GT-38 positive neuron that co-localizes with MAP2. *Scale bars 50 µm.*

Figure S3. Dot-like immunoreactivity pattern in astrocytes stained for pS262 tau. (a-c) A subset of astrocytes showing less confluent and more punctate immunoreactivity for pS262 in CTE, often observed in astrocytic processes. **(a)** Case 6 **(b)** Case 10 **(c)** Case 8. *Scale bars 50 µm.*

Table 1. Demographic and Clinical Summary of Cases								
Case	**Group**	**Age**	**Sex**	**Sport / TBI Exposure**	**Integrated Clinicopathologic Diagnosis**	**PMI**	**Source**	**Anatomical Region Examined**
1	CTE-NC	40s	M	Football	CBD	7 hr	Penn-CNDR	Frontal
2	CTE-NC	60s	M	Football	CBD	3 hr	Penn-CNDR	Frontal
3	CTE-NC	70s	M	Football	DLB	18 hr	Penn-CNDR	Frontal
4	CTE-NC	80s	M	Football	FTLD-TDP	7 hr	Penn-CNDR	Frontal
5	CTE-NC	60s	M	Boxing	CTE	24 hr	GTBIA	Temporal
6	CTE-NC	70s	M	Rugby	CTE	12 hr	GTBIA	Insular
7	CTE-NC	70s	M	Rugby	AD	48 hr	GTBIA	Frontal
8	CTE-NC	70s	M	Rugby	Mixed AD/VaD	48 hr	GTBIA	Frontal
9	CTE-NC	50s	M	Soccer	CTE	Unknown	GTBIA	Insular
10	CTE-NC	80s	M	Soccer	AD	24 hr	GTBIA	Frontal
11	CTE-NC	50s	M	sTBI	Remote TBI - No NDD	108 hr	GTBIA	Frontal
12	CTE-NC	60s	M	sTBI	Remote TBI - No NDD	24 hr	GTBIA	Temporal
13	CTE-NC	70s	M	sTBI	CTE	24 hr	GTBIA	Temporal
14	CTE-NC	70s	M	Mild and Moderate TBI	PDD	7.5 hr	Penn-CNDR	Angular
15	ARTAG, PART	50s	M	No	No NDD	80.5 hr	GTBIA	Frontal
16	ARTAG, PART	80s	M	No	PART	19 hr	Penn-CNDR	Temporal
17	ARTAG, PART	70s	M	No	No NDD	17hr	Penn-CNDR	Temporal
18	PART	60s	M	No	No NDD	11 hr	Penn-CNDR	Temporal
19	ARTAG, Low ADNC	70s	F	No	No NDD	18 hr	Penn-CNDR	Amygdala
20	ARTAG, Low ADNC	70s	F	No	No NDD	19 hr	Penn-CNDR	Amygdala
21	ARTAG, Low ADNC	70s	F	No	No NDD	18 hr	Penn-CNDR	Temporal
22	ARTAG, Low ADNC	80s	M	No	No NDD; CVD	7 hr	Penn-CNDR	Amygdala
23	AD	60s	M	No	AD	13.5 hr	Penn-CNDR	Angular
24	AD	60s	M	No	AD	5 hr	Penn-CNDR	Frontal
25	AD	70s	M	No	AD	4 hr	Penn-CNDR	Angular
26	AD	70s	M	No	AD	8.5 hr	Penn-CNDR	Angular
27	AD	70s	F	No	AD	11 hr	Penn-CNDR	Temporal
28	AD	80s	F	No	AD	6 hr	GTBIA	Cingulate
29	FTLD-Tau	50s	M	No	PiD	11 hr	Penn-CNDR	Temporal
30	FTLD-Tau	60s	M	No	PiD	Unknown	Penn-CNDR	Temporal
31	FTLD-Tau	70s	M	No	PiD	22 hr	Penn-CNDR	Frontal
32	FTLD-Tau	70s	M	No	PiD	4 hr	Penn-CNDR	Angular
33	FTLD-Tau	80s	F	No	PiD	24 hr	Penn-CNDR	Frontal
34	FTLD-Tau	50s	M	No	PiD	10 hr	Penn-CNDR	Frontal
35	FTLD-Tau	70s	M	No	PSP	14 hr	Penn-CNDR	Frontal
36	FTLD-Tau	80s	M	No	PSP	23 hr	Penn-CNDR	Angular
37	FTLD-Tau	70s	M	No	PSP	23 hr	Penn-CNDR	Frontal
38	FTLD-Tau	60s	M	No	PSP	13 hr	Penn-CNDR	Angular
39	FTLD-Tau	70s	M	No	PSP	17 hr	Penn-CNDR	Temporal
40	FTLD-Tau	70s	M	No	PSP	19 hr	Penn-CNDR	Frontal
41	FTLD-Tau	60s	F	No	CBD	16 hr	Penn-CNDR	Temporal
42	FTLD-Tau	70s	M	No	CBD	11 hr	Penn-CNDR	Angular
43	FTLD-Tau	50s	M	No	CBD	41 hr	Penn-CNDR	Frontal
44	FTLD-Tau	70s	M	No	CBD	7.5 hr	Penn-CNDR	Temporal
45	FTLD-Tau	80s	M	No	CBD	11 hr	Penn-CNDR	Frontal
46	FTLD-Tau	70s	M	No	CBD	5 hr	Penn-CNDR	Frontal

Key: *PMI: Post-mortem interval; CTE-NC: Chronic traumatic encephalopathy neuropathologic change; CBD: Corticobasal degeneration; DLB: Dementia with Lewy bodies; FTLD-TDP: Frontotemporal lobar degeneration with TDP-43 inclusions; AD: Alzheimer's Disease; VaD: Vascular dementia; TBI: Traumatic brain injury; NDD: Neurodegenerative disease; PDD: Parkinson Disease Dementia; ARTAG: Aging-related tau astrogliopathy; PART: Primary age-related tauopathy; ADNC: Alzheimer's Disease neuropathologic change; CVD: Cerebrovascular disease; PiD: Pick's Disease; PSP: Progressive supranuclear palsy; sTBI: Single moderate or severe TBI; GTBIA: Glasgow TBI Archive; Penn-CNDR: University of Pennsylvania Center for Neurodegenerative Disease Research*

Table 2. Antibody Details

Antibody	Antigen	Monoclonal / Polyclonal (Species)	Antigen Retrieval	Dilution	Source
RD3	Three microtubule binding domain repeat (3R) tau	Monoclonal (Ms)	FA & Citrate Buffer (pH 6.0)	DAB: 1:12000 (Penn-CNDR) 1:6000 (GTBIA)	Millipore Sigma (Burlington, MA)
RD4	Four microtubule binding domain repeat (4R) tau	Monoclonal (Ms)	FA & Citrate Buffer (pH 6.0)	DAB: 1:400	Millipore Sigma (Burlington, MA)
PHF1	Tau pS396 and pS404	Monoclonal (Ms)	Tris-EDTA Buffer (pH 8.0)	DAB: 1:1000 IF: 1:100	Dr. Peter Davies (Albert Einstein College of Medicine, NY)
CP13	Tau pS202	Monoclonal (Ms)	Tris-EDTA Buffer (pH 8.0)	DAB: 1:1000	Dr. Peter Davies (Albert Einstein College of Medicine, NY)
Tau pS262	Tau pS262	Polyclonal (Rb)	Citrate Buffer (pH 6.0)	DAB: 1:1000	Millipore Sigma (Burlington, MA)
AT100	Tau pS212 and pT214	Monoclonal (Ms)	Tris-EDTA Buffer (pH 8.0)	DAB: 1:1000	ThermoFisher (Waltham, MA)
Tau-C3	Caspase-cleaved tau, truncated at Asp421	Monoclonal (Ms)	Citrate Buffer (pH 6.0)	DAB: 1:1000	Millipore Sigma (Burlington, MA)
GT-7	Conformation dependent tau	Monoclonal (Ms)	FA & Citrate Buffer (pH 6.0)	DAB: 1:1000	Penn CNDR (Philadelphia, PA)
GT-38	Conformation dependent tau	Monoclonal (Ms)	FA & Citrate Buffer (pH 6.0)	DAB: 1:1000 IF: 1: 1:100	Penn CNDR (Philadelphia, PA)
MAP2	Microtubule-associated protein 2	Polyclonal (Ck)	Tris-EDTA Buffer (pH 8.0)	IF: 1:200	Abcam (Cambridge, UK)
GFAP	Glial fibrillary acid protein, astrocyte-specific	Monoclonal (Rb)	Tris-EDTA Buffer (pH 8.0)	IF: 1:200	Abcam (Cambridge, UK)

FA denotes 5 minutes of formic acid pre-treatment; Citrate versus Tris-EDTA buffer denotes pretreatment with heat/pressure submerged in respective buffer. DAB: 3,3'-Diaminobenzidine visualization protocol; IF: immunofluorescence protocol; Rb: rabbit; Ms: mouse; Ck: Chicken.

Table 3. Tau Phenotype Semi-Quantitative Scores – 3R, 4R and Conformation								
Case	**3R**		**4R**		**GT-7**		**GT-38**	
	TSA	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**
1 – CTE-NC	-	++	+++	+++	-	++	-	++
2 - CTE-NC	-	++	+++	+++	-	++	-	++
3 - CTE-NC	-	+++	+++	+++	-	+++	-	+++
4 - CTE-NC	+	++	+++	+++	-	++	+	++
5 - CTE-NC	-	+++	+++	+++	-	+++	-	+++
6 - CTE-NC	-	+++	+++	+++	-	+++	-	+++
7 - CTE-NC	+	+++	+++	+++	-	+++	-	+++
8 - CTE-NC	-	+++	+++	+++	+	+++	+	+++
9 - CTE-NC	x	x	x	x	x	x	x	x
10 - CTE-NC	-	+++	+++	+++	-	+++	-	+++
11 - CTE-NC	x	x	x	x	-	+++	-	+++
12 - CTE-NC	-	++	++	++	-	++	-	++
13 - CTE-NC	-	+++	++	++	+	+++	+	+++
14 - CTE-NC	-	+++	+++	+++	+	++	-	++
	TSA	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**
15 - ARTAG, PART	x	x	x	x	-	+++	-	+++
16- ARTAG, PART	-	+	+++	+++	-	+++	-	+++
17- ARTAG, PART	-	+++	+++	+++	-	+++	-	+++
18 - PART	N/A	++	N/A	++	N/A	++	N/A	++
19 - ARTAG, Low AD-NC	-	+++	+++	+++	-	++	-	+++
20 - ARTAG, Low AD-NC	+	++	+++	+++	+	+++	+	+++
21 - ARTAG, Low AD-NC	-	+++	+++	+++	-	+++	-	+++
22 - ARTAG, Low AD-NC	-	+++	+++	+++	-	+++	-	+++
	NFT		**NFT**		**NFT**		**NFT**	
23 - AD	+++		+++		+++		+++	
24 - AD	+++		+++		+++		+++	
25 - AD	+++		+++		+++		+++	
26 - AD	+++		+++		+++		+++	
27 - AD	+++		+++		+++		+++	
28 - AD	++		++		+++		+++	
	Pick Bodies	**Ramified Astro**	**Pick Bodies**	**Ramified Astro**	**Pick Bodies**	**Ramified Astro**	**Pick Bodies**	**Ramified Astro**
29 - PiD	+++	+++	-	++	-	-	-	-
30 - PiD	+++	N/A	-	N/A	-	N/A	-	N/A
31 - PiD	+++	+++	-	-	-	-	-	-
32 - PiD	+++	N/A	-	N/A	-	N/A	-	N/A
33 - PiD	+++	N/A	-	N/A	-	N/A	-	N/A
34 - PiD	+++	+++	-	-	-	-	-	-
	Tufted Astrocytes		**Tufted Astrocytes**		**Tufted Astrocytes**		**Tufted Astrocytes**	
35 - PSP	-		+++		-		-	
36 - PSP	-		+++		-		-	
37 - PSP	-		++		-		-	
38 - PSP	-		+++		-		-	
39 - PSP	-		++		-		-	
40 - PSP	-		+++		-		-	
	Astrocytic Plaques		**Astrocytic Plaques**		**Astrocytic Plaques**		**Astrocytic Plaques**	
41 - CBD	-		+++		-		-	
42 - CBD	-		+++		-		-	
43 - CBD	-		+++		-		-	
44 - CBD	-		+++		-		-	
45 - CBD	-		+++		-		-	
46 - CBD	-		+++		-		-	

Semi-quantitative Scoring: - absent, or nearly absent (≤5% concordance with extent of PHF1); + minimal (5-30% concordance); ++ moderate (30-70% concordance); +++ extensive (>70% concordance); X: No immunoreactivity

CTE-NC: Chronic traumatic encephalopathy neuropathologic change; TSA: thorn-shaped astrocytes; NFT: neurofibrillary tangles; ARTAG: Aging-related tau astrogliopathy; PART: Primary age-related tauopathy; AD-NC: Alzheimer's Disease neuropathologic change; AD: Alzheimer's Disease; FTLD-Tau: Frontotemporal lobar degeneration tauopathies; N/A: not applicable.

Table 4. Tau Phenotype Semi-Quantitative Scores – Post-Translational Modifications										
Case	**PHF-1**		**CP13**		**AT100**		**pS262**		**Tau-C3**	
	TSA	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**
1 - CTE-NC	+++	+++	+++	+++	+	+++	+++	+++	-	+
2 - CTE-NC	+++	+++	+++	+++	+++	+++	+++	+++	-	+
3 - CTE-NC	+++	+++	+++	+++	+++	+++	+++	+++	-	-
4 - CTE-NC	+++	+++	+++	+++	++	++	+++	+++	-	++
5 - CTE-NC	+++	+++	+++	+++	+++	+++	+	+++	-	+
6 - CTE-NC	+++	+++	+++	+++	+++	+++	++	+++	-	+
7 - CTE-NC	+++	+++	+++	+++	+++	+++	+	+++	-	+
8 - CTE-NC	+++	+++	+++	+++	+++	+++	++	+++	-	+
9 - CTE-NC	+++	+++	+++	+++	+++	+++	-	+	x	x
10 - CTE-NC	+++	+++	+++	+++	+++	+++	++	+++	+	++
11 - CTE-NC	+++	+++	+++	+++	+++	+++	-	+++	-	+
12 - CTE-NC	+++	+++	+++	+++	++	+++	-	+++	-	+
13 - CTE-NC	+++	+++	+++	+++	+++	+++	++	+++	-	++
14 - CTE-NC	+++	+++	+++	+++	+++	+++	+++	+++	-	+
	TSA	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**	**TSA**	**NFT**
15 - ARTAG, PART	+++	+++	+++	+++	+++	+++	+	++	+	++
16.- ARTAG, PART	+++	+++	+++	+++	+++	+++	++	+++	-	+++
17.- ARTAG, PART	+++	+++	+++	+++	+++	+++	+	+++	-	+
18 - PART	N/A	+++	N/A	+++	N/A	++	N/A	++	N/A	-
19 - ARTAG, Low AD-NC	+++	+++	+++	+++	+++	+++	+++	+++	-	+
20 - ARTAG, Low AD-NC	+++	+++	+++	+++	+++	+++	+++	+++	-	++
21 - ARTAG, Low AD-NC	+++	+++	+++	+++	+++	+++	++	+++	-	-
22 - ARTAG, Low AD-NC	+++	+++	+++	+++	+++	+++	+++	+++	-	-
	NFT		**NFT**		**NFT**		**NFT**		**NFT**	
23 - AD	+++		+++		++		+++		++	
24 - AD	+++		+++		+++		+++		++	
25 - AD	+++		+++		+++		+++		+	
26 - AD	+++		+++		+++		+++		+	
27 - AD	+++		+++		+++		+++		++	
28 - AD	+++		+++		+++		+++		+++	
	Pick Bodies	**Ramified Astro**	**Pick Bodies**	**Ramified Astro**	**Pick Bodies**	**Ramified Astro**	**Pick Bodies**	**Ramified Astro**	**Pick Bodies**	**Ramified Astro**
29 - PiD	+++	+++	+++	+++	+++	+++	++	+	++	-
30 - PiD	+++	N/A	+++	N/A	+++	N/A	-	N/A	++	N/A
31 - PiD	+++	+++	+++	+++	+++	+++	-	-	-	-
32 - PiD	+++	N/A	+++	N/A	+++	N/A	-	N/A	-	N/A
33 - PiD	+++	N/A	+++	N/A	+++	N/A	-	N/A	-	N/A
34 - PiD	+++	+++	+++	+++	+++	+++	-	-	-	-
	Tufted Astrocytes		**Tufted Astrocytes**		**Tufted Astrocytes**		**Tufted Astrocytes**		**Tufted Astrocytes**	
35 - PSP	+++		+++		++		++		+	
36 - PSP	+++		+++		+++		+		+++	
37 - PSP	+++		+++		+++		+		-	
38 - PSP	+++		+++		+++		+		-	
39 - PSP	+++		+++		++		-		-	
40 - PSP	+++		+++		+++		++		-	
	Astrocytic Plaques		**Astrocytic Plaques**		**Astrocytic Plaques**		**Astrocytic Plaques**		**Astrocytic Plaques**	
41 - CBD	+++		+++		+		+		-	
42 - CBD	+++		+++		+++		-		-	
43 - CBD	+++		+++		++		-		-	
44 - CBD	+++		+++		++		++		-	
45 - CBD	+++		+++		+++		+		-	
46 - CBD	+++		+++		+++		+++		-	

Semi-quantitative Scoring: - absent, or nearly absent (≤5% concordance with extent of PHF1); + minimal (5-30% concordance); ++ moderate (30-70% concordance); +++ extensive (>70% concordance); X: No immunoreactivity

CTE-NC: Chronic traumatic encephalopathy neuropathologic change; TSA: thorn-shaped astrocytes; NFT: neurofibrillary tangles; ARTAG: Aging-related tau astrogliopathy; PART: Primary age-related tauopathy; AD-NC: Alzheimer's Disease neuropathologic change; AD: Alzheimer's Disease; FTLD-Tau: Frontotemporal lobar degeneration tauopathies.

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