

Chronic traumatic encephalopathy pathology in a neurodegenerative disorders brain bank

Kevin F. Bieniek^{1,2} · Owen A. Ross¹ · Kerry A. Cormier³ · Ronald L. Walton¹ · Alexandra Soto-Ortolaza¹ · Amelia E. Johnston⁴ · Pamela DeSaro⁴ · Kevin B. Boylan⁴ · Neill R. Graff-Radford⁴ · Zbigniew K. Wszolek⁴ · Rosa Rademakers¹ · Bradley F. Boeve⁵ · Ann C. McKee^{3,6} · Dennis W. Dickson¹

Received: 28 September 2015 / Revised: 23 October 2015 / Accepted: 25 October 2015 / Published online: 30 October 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative disorder linked to repetitive traumatic brain injury (TBI) and characterized by deposition of hyperphosphorylated tau at the depths of sulci. We sought to determine the presence of CTE pathology in a brain bank for neurodegenerative disorders for individuals with and without a history of contact sports participation. Available medical records of 1721 men were reviewed for evidence of past history of injury or participation in contact sports. Subsequently, cerebral cortical samples were processed for tau immunohistochemistry in cases with a documented history of sports exposure as well as age- and disease-matched men and women without such exposure. For cases with available frozen tissue, genetic analysis was performed for variants in *APOE*, *MAPT*, and *TMEM106B*.

Immunohistochemistry revealed 21 of 66 former athletes had cortical tau pathology consistent with CTE. CTE pathology was not detected in 198 individuals without exposure to contact sports, including 33 individuals with documented single-incident TBI sustained from falls, motor vehicle accidents, domestic violence, or assaults. Among those exposed to contact sports, those with CTE pathology did not differ from those without CTE pathology with respect to noted clinicopathologic features. There were no significant differences in genetic variants for those with CTE pathology, but we observed a slight increase in *MAPT* H1 haplotype, and there tended to be fewer homozygous carriers of the protective *TMEM106B* rs3173615 minor allele in those with sports exposure and CTE pathology compared to those without CTE pathology. In conclusion, this study has identified a small, yet significant, subset of individuals with neurodegenerative disorders and concomitant CTE pathology. CTE pathology was only detected in individuals with documented participation in contact sports. Exposure to contact sports was the greatest risk factor for CTE pathology. Future studies addressing clinical correlates of CTE pathology are needed.

Electronic supplementary material The online version of this article (doi:10.1007/s00401-015-1502-4) contains supplementary material, which is available to authorized users.

✉ Dennis W. Dickson
dickson.dennis@mayo.edu

¹ Department of Neuroscience, Mayo Clinic, 4500 San Pablo Rd., Jacksonville, FL 32224, USA

² Mayo Graduate School, Mayo Clinic, 200 First St., Rochester, MN 55905, USA

³ VA Boston HealthCare System, 150 South Huntington Ave, Boston, MA 02130, USA

⁴ Department of Neurology, Mayo Clinic, 4500 San Pablo Rd., Jacksonville, FL 32224, USA

⁵ Department of Neurology, Mayo Clinic, 200 First St. SW, Rochester, MN 55905, USA

⁶ Department of Neurology and Pathology, Boston University Alzheimer's Disease Center and CTE Program, Boston University School of Medicine, Boston, MA 02118, USA

Keywords Chronic traumatic encephalopathy · Traumatic brain injury · Sports · Microtubule-associated protein tau · Brain bank

Introduction

Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative disorder linked to repetitive traumatic brain injury [5, 8, 9, 35]. First described in boxers as “dementia pugilistica” [4], CTE is now used more widely to describe a neurodegenerative disorder associated with a variety of

contact sports, as well as traumatic brain injury sustained in military training and combat [6, 11, 23]. Clinical symptoms of CTE usually occur 8–10 years after exposure to repetitive brain injury, and can progress from headaches, loss of attention and short-term memory loss, to behavioral changes, rage dyscontrol, depression, suicidality, dementia, and gait abnormalities [20]. Two major clinical variants of CTE have been described, with younger patients exhibiting more pronounced behavioral or mood disturbances and older patients exhibiting cognitive impairment that overlaps with other age-associated cognitive disorders, such as Alzheimer's disease (AD) [33].

Neuropathologically, CTE is defined by deposition of hyperphosphorylated tau in neurofibrillary tangles, threads and astrocytes, as well as variable axonal and transactive response DNA-binding protein 43 (TDP-43) pathology [20, 22]. Tau deposition is typically focal and characteristically most severe at the depths of cerebral sulci, often surrounding penetrating cortical blood vessels. Cross-sectional studies in experienced athletes suggest CTE pathology progresses from focal deposits ("epicenters") in the neocortex to multifocal cortical lesions and to later involvement of medial temporal lobe, basal ganglia, diencephalon and brainstem. A staging scheme has been proposed, with cortical pathology in Stages I and II, and more extensive subcortical pathology in Stages III and IV [23].

CTE pathology can be accompanied by other neurodegenerative processes. Subsets of patients with CTE have amyloid- β , TDP-43 or α -synuclein pathology [16, 23, 31, 32]. CTE pathology has also been reported in patients with amyotrophic lateral sclerosis (ALS) [7]. TDP-43 pathology is detected in up to 80 % of CTE cases, and pathologically confirmed ALS is reported in approximately 12 % of CTE cases [15, 22, 23].

Between 1982 and 2008, over 160 million men and women participated in sports in the USA at either the high school or collegiate level [3]. The proportion of this cohort at risk for developing CTE is unknown. While current studies have focused on characterizing CTE in defined cohorts of mostly professional athletes and military veterans, there are no studies that have examined the frequency of CTE in unselected individuals from the general population. As an initial effort to address this problem, we screened for CTE pathology in brains of individuals with exposure to contact sports and matched controls without such exposure, all derived from a brain bank for neurodegenerative disorders.

Methods

Case material

The Mayo Clinic brain bank in Jacksonville, FL is an IRB-approved brain bank that holds brains of individuals with a

range of neurodegenerative disorders, as well as a smaller number of neurologically normal controls. In addition to formalin-fixed, paraffin-embedded and frozen brain tissue, variable medical records are available for study. Between 1997 and 2014, 4711 cases were acquired by the brain bank. All autopsies were performed after consent from the legal next-of-kin or an individual holding power-of-attorney. The inclusion criteria for this study were presence of paraffin-embedded tissue, at least minimal medical documentation, and male sex. The major exclusion criteria were a neuropathologic diagnosis of progressive supranuclear palsy, corticobasal degeneration, Pick's disease, and cases with a mutation in the microtubule-associated protein tau gene (*MAPT*). Atypical tauopathies, including primary age-related tauopathy, which did not meet diagnostic criteria for progressive supranuclear palsy, corticobasal degeneration, or Pick's disease and lacked a known *MAPT* mutation, were included in this study (FTLD-tau). The resulting cohort consisted of 1721 cases, of which available medical records were queried for information regarding participation in contact sports. These cases were received from the following sources: State of Florida Alzheimer's Disease Initiative ($N = 611$), Mayo Clinic Morris K. Udall Center of Excellence for Parkinson's Disease ($N = 345$), Mayo Clinic Jacksonville Alzheimer's Disease Research Center ($N = 191$), CurePSP: Society for PSP | CBD and Related Disorders ($N = 177$), consultation cases ($N = 161$), Mayo Clinic Jacksonville hospital autopsy cases ($N = 139$), Mayo Clinic Jacksonville ALS Clinic ($N = 39$), Einstein Aging Study ($N = 35$), Mayo Clinic Rochester Alzheimer's Disease Patient Registry ($N = 12$), and Mayo Clinic Alzheimer's Disease Research Center ($N = 11$). The depth of the clinical information on head trauma (and subsequently contact sports exposure) varied between these sources.

Pertinent clinical information derived from the available records included gender, age at symptom onset, symptom duration, age at death, height, education, family history of dementia or parkinsonism, alcohol use (including amount), tobacco use (including amount), military service, contact sports participation, the level of sports participation, and documented head trauma associated with sports participation. Exposure to non-sports-related head trauma, including falls with head injury, motor vehicle accidents, violent assaults, and domestic abuse was recorded as well. Contact sports were defined as organized athletics with the potential for repetitive traumatic brain injury to the participants, including American football, boxing, wrestling, rugby, soccer, ice hockey, basketball, baseball, martial arts, and rodeo. Non-contact sports that were excluded from the contact sports exposure group included track and field, cross country, swimming, tennis, and golf. Online searches of obituaries were conducted to validate and supplement clinical records for individuals with contact sports exposure and controls using full name, date of birth and date of death.

Neuropathologic controls included men without documented exposure to contact sports selected from the 1721 men identified in the screen of medical records. They were matched on a two-to-one ratio to those with exposure to contact sports with respect to age at death and comorbid pathological processes. Sixty-six women without documented sports exposure were similarly matched. Clinical controls ($N = 408$) for genetic analysis were evaluated by a neurologist at Mayo Clinic Florida and had no neurological disorder or family history of neurological disease. All were Caucasian males and the median age at the time of blood draw was 68 years.

Neuropathologic assessment

Most of the 1721 brains were received with the left hemisphere fixed in 10 % formalin and the right hemisphere frozen ($N = 1257$). Formalin-fixed brains underwent systematic and standardized sampling, and neuropathologic evaluation by a single neuropathologist (DWD). Regions sampled on all cases processed by the Mayo Clinic brain bank included neocortex (6×; primary and association cortices), hippocampus (2×), basal forebrain, striatum, thalamus, midbrain, pons, medulla, and cerebellum (2×). Neuropathologic evaluation included review of histologic stains (hematoxylin and eosin), thioflavin S fluorescent microscopy, and select immunohistochemistry at Dr. Dickson's discretion (i.e., α -synuclein and TDP-43). Braak Neurofibrillary Tangle Stage and Thal Phase were assigned using thioflavin S slides and published criteria (Supplemental Table 1) [2, 34].

To assess CTE pathology, 5- μ m-thick sections from convexity cerebral cortices [dorsolateral middle frontal gyrus (Brodmann area 46/9) and inferior parietal lobule (Brodmann area 39/40)] were processed for immunohistochemistry. Following deparaffinization in xylene and reagent alcohol, antigen retrieval was performed by steaming slides in distilled water for 30 min. All immunohistochemistry was conducted using a DAKO Autostainer and DAKO EnVision™ + reagents. The tau antibody used was CP13 (mouse monoclonal; 1:1000; a gift from Dr. Peter Davies, Feinstein Institute for Medical Research). The TDP-43 antibody pS409/410 (mouse monoclonal; 1:5000; CosmoBio USA, Inc.) was used for immunohistochemistry in the posterior hippocampus. Immunostained slides were counterstained with hematoxylin and coverslipped. For cases suspected of having CTE pathology based on screening of standard (1.5 × 5.5 cm) histologic slides, coronal slabs of formalin-fixed tissue at the level of the infundibulum were sectioned at 50 μ m thickness on a sliding microtome and immunostained as free-floating sections with CP13 [23]. Cases with CTE pathology were staged using whole mount coronal slides according to the McKee staging scheme (Table 1).

Genetic analysis

Genomic DNA was extracted from cerebellum of frozen brains of men with exposure to contact sports or from blood of neurologically normal men. Genotyping was performed with TaqMan Allelic Discrimination Assay on an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems). A single SNP (rs1052553) was used to determine *MAPT* haplotype and *TMEM106B* (rs3173615) and two SNPs (rs7412 and rs429358) were used to determine *APOE* genotype. Five μ L reactions consisting of 20 ng of DNA, 1× TaqMan genotyping master mix and 1× TaqMan SNP genotyping assay with appropriate genomic controls were used to genotype the four variants. Thermal cycling conditions consisted of one enzyme activation step at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Genotype calls were performed with SDS 2.2.2 software.

Statistical methods

Statistical analysis was conducted using SigmaPlot software (version 11.0, Systat Software Inc.). Rank sum test was performed when comparing continuous quantitative variables, while the Chi-square test was performed when comparing categorical variables between cases and controls, and between cases with and without CTE pathology. Fisher's exact test was used to compare presence or absence of CTE pathology in former football players due to small sample size. A statistically significant difference was considered for two-sided P values <0.05.

Results

Retrospective review of available records of 1721 men identified 66 patients with history of exposure to contact sports (Fig. 1). Demographic and clinical features of this cohort are summarized in Table 1. Most (54 men) participated in a single sport (34 American football, 8 boxers, 7 baseball, 1 basketball, 1 ice hockey, 1 soccer, 1 rodeo and 1 martial artist), but nine participated in multiple sports [football plus: baseball (1), basketball (2), basketball and baseball (2), boxing (1), wrestling (1), rugby (1), and soccer (1)]. Three subjects had comments in medical records ("possible concussion while playing sports as a youngster," "three concussions with being not fully conscious while doing sports," and "brief loss of consciousness playing sports") suggestive of contact sports, but without details on the specific sport.

Sixty-five of the 66 men were Caucasian (one Hispanic). The average level of education ($N = 56$) was 15.9 years. The average height ($N = 35$) was 176 cm.

Table 1 Clinical and demographic features of men with contact sports exposure

Case #	CTE	Sport	Sport level	Veteran	Education	FHx	Clinical Dx	Path Dx	Onset	Death	Duration	Alcohol	Tobacco
1	I	Bk	HS	-	?	-	ALS	ALS	45	50	5	+	-
2	I	F	HS	-	18	+	FTD	FTLD-FUS	37	41	4	+	-
3	I	F	HS	+	?	+	ALS	ALS	45	47	2	?	?
4	I	F	HS	+	16	-	PDD	AD + LBD	65	79	14	+	+
5	I	F	HS, Co	-	16	-	DLB	LBD	66	71	5	+	+
6	I	F/Bk/Bb	HS, Co[Bb], sPro[Bb]	+	16	-	MSA	AD + LBD	63	74	11	?	?
7	I	F/W	HS, Co, Pro[W]	+	16	+	FTD/CVA	AD	?	80	?	+	+
8	II	?	?	+	16	+	AD	AD	83	87	4	-	-
9	II	F	?	-	?	-	ALS	ALS	30	45	15	+	+
10	II	Bb	sPro	+	12	-	NCI	AD	NA	87	NA	+	+
11	II	Bo	Am	-	5	+	AD	AD	75	87	12	+	-
12	II	F	HS, Co	-	18	-	NCI	AD	NA	68	NA	+	+
13	II	F	HS, Co	-	16	+	NCI	Normal	NA	51	NA	-	+
14	II	F	HS, Co	+	15	-	PSP	LBD	73	79	6	-	-
15	III	F	HS	-	17	+	AD/PD	AD + LBD	53	75	22	+	+
16	III	F	HS	+	14	+	DLB	AD + LBD	70	78	8	+	+
17	III	F	HS, Co, Pro	+	16	+	VaD	FTLD-tau	67	70	3	-	-
18	III	F/Ru	?	+	16	+	PDD	LBD	65	81	16	+	+
19	III	F/Bb	Co	-	16	-	AD	FTLD-tau	75	82	7	+	-
20	IV	Bo	Pro	+	5	-	AD v DLB	AD	77	93	16	-	-
21	IV	F/Bk/Bb	HS, Co, sPro[Bb]	+	16	+	PSP	FTLD-tau	60	81	21	?	?
22	-	Bb	Co, sPro	-	18	-	FTD-MND	FTLD-TDP	?	71	?	-	-
23	-	Bb	Co, sPro	+	16	+	DLB	AD + LBD	62	76	14	-	-
24	-	Bb	HS	-	16	-	PD	LBD	45	73	28	?	?
25	-	Bb	HS	-	12	+	AD/aphasia	AD	76	86	10	+	+
26	-	Bb	HS	+	18	-	CBS v FTD	AD + LBD	56	65	9	+	+
27	-	Bb	HS, Co	+	16	+	FTD v DLB	VaD	67	76	9	+	+
28	-	Bo	Am	-	14	+	AD	AD	55	71	16	+	+
29	-	Bo	Am	+	12	+	AD	AD	63	74	11	-	-
30	-	Bo	Am	+	12	+	AD v DLB	AD	70	80	10	-	+
31	-	Bo	Am	+	20	+	AD	AD	67	73	6	+	+
32	-	Bo	Am	+	14	-	AD	AD	62	68	6	?	?
33	-	Bo	?	+	?	-	AD v DLB	AD + LBD	63	70	7	-	+
34	-	F	Co	-	16	+	AD v FTD	AD	57	65	8	+	+
35	-	F	Co	+	16	+	PDD	AD + LBD	63	74	11	-	-

Table 1 continued

Case #	CTE	Sport	Sport level	Veteran	Education	FHx	Clinical Dx	Path Dx	Onset	Death	Duration	Alcohol	Tobacco
36	-	F	HS	-	20	-	CBS	AD	59	67	8	+	-
37	-	F	HS	-	18	-	FTD	AD	69	78	9	?	?
38	-	F	HS	-	16	-	AD v DLB	AD	56	64	8	+	+
39	-	F	HS	-	20	+	AD	AD	53	61	8	+	+
40	-	F	HS	-	?	-	AD	AD + LBD	74	78	4	+	-
41	-	F	HS	-	18	-	AD v DLB	AD + LBD	66	77	11	+	-
42	-	F	HS	+	12	-	AD v DLB	AD + LBD	78	87	9	+	+
43	-	F	HS	+	?	-	ALS	ALS	72	74	2	-	?
44	-	F	HS	+	16	-	AD	FTLD-TDP	48	56	8	-	-
45	-	F	HS	+	18	+	AD/VaD	AD	70	83	13	+	+
46	-	F	HS	+	14	+	AD	AD	60	81	21	+	+
47	-	F	HS	+	21	+	AD	AD	70	80	10	+	+
48	-	F	HS, Co	-	22	-	FTD	AD	69	73	4	+	-
49	-	F	HS, Co	-	16	+	AD/VaD	AD + LBD	52	60	8	+	+
50	-	F	HS, Co	+	18	-	DLB	LBD	85	90	5	?	?
51	-	F	HS, Co	+	16	+	AD	FTLD-TDP	80	89	9	+	-
52	-	F	HS, Co	+	16	-	AD	AD	55	59	4	+	-
53	-	F	?	-	?	-	AD	AD	73	78	5	+	+
54	-	F	?	-	?	-	PDD	LBD	73	93	20	+	+
55	-	F	?	-	16	-	MSA	MSA	69	76	7	-	-
56	-	F	?	+	16	-	NCI	ATP	NA	64	NA	+	-
57	-	F/Bk	HS	-	16	+	PDD	AD + LBD	49	57	8	-	-
58	-	F/Bk	HS, Co[Bk]	-	18	-	AD	AD	63	73	10	+	+
59	-	F/Bo	?	+	14	-	AD	AD + LBD	69	79	10	+	+
60	-	F/S	HS	+	20	+	AD	AD	70	79	9	-	+
61	-	H	?	+	?	+	AD v DLB	AD + LBD	68	78	10	+	-
62	-	MA	?	+	22	+	AD	AD	76	80	4	+	-
63	-	Ro	?	-	12	-	AD	AD + LBD	64	70	6	+	-
64	-	S	Am	-	?	-	AD	AD	66	73	7	+	-
65	-	?	?	-	18	-	AD/PD	AD + LBD	72	79	7	+	+
66	-	?	?	-	14	-	MSA v PDD	LBD	71	79	8	+	+

AD Alzheimer's disease, ALS amyotrophic lateral sclerosis, Am amateur, ATP Alzheimer-type pathology (does not meet criteria for AD), Bb baseball, Bk basketball, Bo boxing, Co college, CBS corticobasal syndrome, CTE CTE Stage (-, no CTE), CVA cerebrovascular accident, DLB dementia with Lewy bodies, Dx diagnosis, F football, FHx family history of dementia or parkinsonism, FTD frontotemporal dementia, FTLD-FUS frontotemporal lobar degeneration with fused in sarcoma protein, FTLD-tau frontotemporal lobar degeneration with atypical tau protein, FTLD-TDP frontotemporal lobar degeneration with TDP-43 protein, H hockey, HS high school, LBD Lewy body disease, MA martial arts, MND motor neuron disease, MSA multiple systems atrophy, NA not applicable, NCI no cognitive impairment, PD Parkinson's disease, PDD Parkinson's disease, PDP progressive supranuclear palsy, Ro rodeo, Ru rugby, S soccer, sPro semi-professional, VaD vascular dementia, v versus, W: wrestling, ? unknown

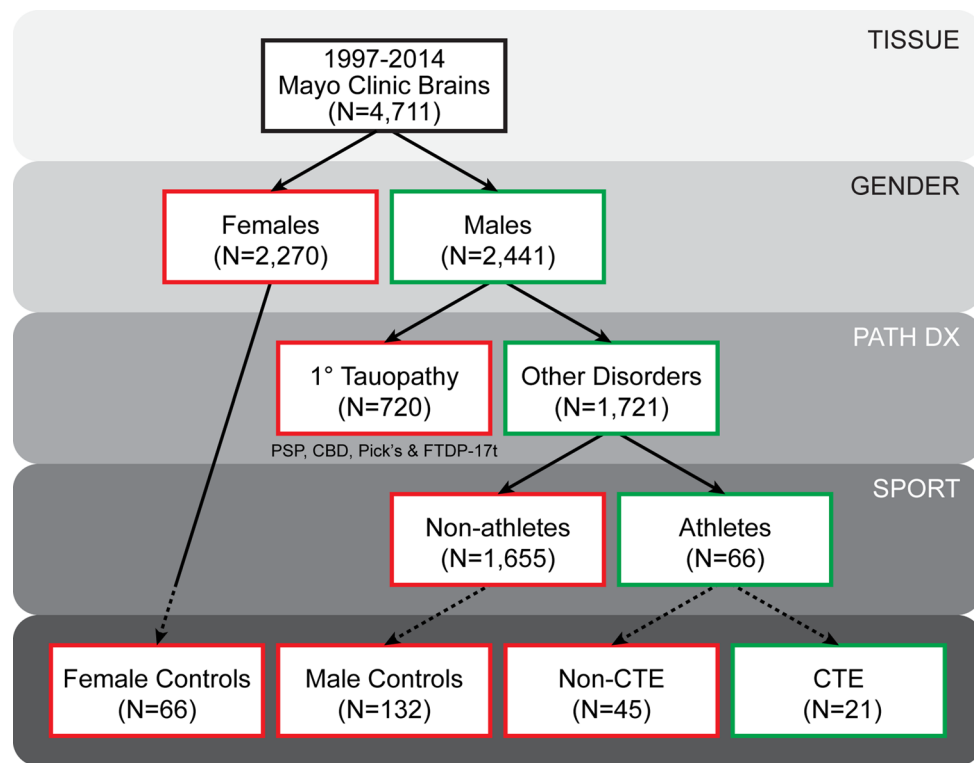


Fig. 1 Selection criteria for Mayo Clinic Florida cohort. There are 4711 brains with available formalin-fixed tissue recruited between the years 1997 and 2014. Of these 4711 brains, 2441 were male and 1721 males did not have a prior neuropathologic diagnosis (Path Dx) of a primary tauopathy, namely progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (Pick's), or frontotemporal lobar degeneration with parkinsonism with a known *MAPT* mutation (FTDP-17t). Available clinical records of these 1721 males

were queried for any description of involvement in a contact sport, and 66 individuals did have notation regarding former athletics participation. Screening cortical tissue via tau immunohistochemistry (dashed lines) identified 21 cases with CTE pathology and 45 cases that failed to meet study standards of CTE pathology. In addition, tau immunohistochemistry was also performed on 66 female and 132 male age- and disease-matched (Path Dx) controls without documentation of sports involvement

Alcohol usage ($N = 59$) was endorsed by 43 individuals and denied by 16 individuals. Of the 43 former athletes that endorsed alcohol use, 11 individuals were noted to have consumed alcohol minimally or periodically, 24 individuals on average consumed less than 2 alcoholic drinks per day, and 8 individuals were described as 'heavy' drinkers at some point throughout their documented clinical course or consumed 2 drinks or more per day. Tobacco usage ($N = 58$) was endorsed by 33 individuals and denied by 25 individuals. Of the 33 former athletes that endorsed tobacco use, 5 smoked for 0–10 pack years, 17 smoked for 11–30 pack years, and 11 smoked for 30 or more pack years. Additionally, 35 men served in the United States Armed Forces. A control cohort of 198 disease-matched individuals (132 men and 66 women) was selected based upon age at death. None of these individuals had documented involvement in contact sports; however, 33 had history of head trauma, including falls ($N = 14$), motor vehicle accidents ($N = 10$), violent assaults ($N = 4$), domestic abuse ($N = 3$), and 2 unspecified head trauma.

Tau immunohistochemistry was performed on sections from the dorsolateral frontal and parietal lobes. Of the 66 men with exposure to contact sports, 21 (32 %) had tau pathology consistent with CTE (Fig. 2). This was characterized by subpial thorn-shaped astrocytes (TSA) at the depths of sulci; neurofibrillary tangles (NFT), pretangles and threads in superficial cortical layers; as well as perivascular epicenters in the cortical ribbon at the depths of sulci composed of TSA, NFT and threads [17]. Tau immunohistochemistry uncovered select characteristic features, including pretangles and TSA, which were not observed on thioflavin S microscopy. The overall pattern of tau pathology was patchy and best demonstrated on the whole hemisphere coronal slides (Fig. 3). If Alzheimer-type pathological change was present, it was distinguished by more uniform involvement of lamina 3 and 5 of the cortical ribbon with no overt predilection for depths of sulci (Fig. 4). CTE tau pathology was observed in both frontal and parietal cortices at a similar frequency in 17 cases, only in the parietal cortex in 2 cases, and only in the frontal cortex in 2 cases. Regarding CTE pathologic distribution, 7 cases were

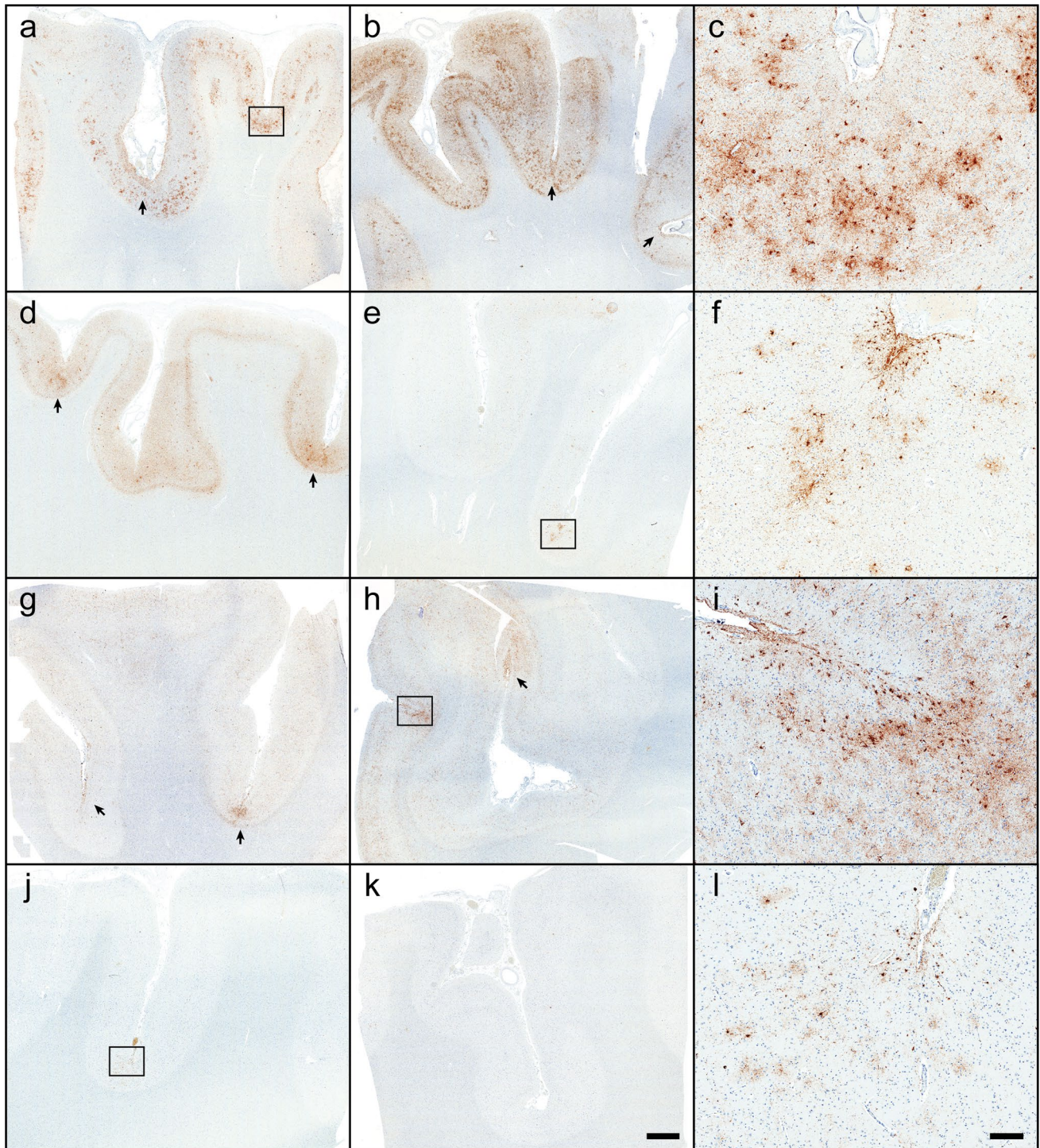


Fig. 2 Chronic traumatic encephalopathy in the Mayo Clinic Florida cohort. Tau immunohistochemistry on tissue from the frontal cortex (**a, d, g, j**) and parietal cortex (**b, e, h, k**) in 66 athletes and 198 controls revealed CTE pathology in 21 cases, including a former boxer (**a–c** Case #20), a former high school and collegiate American football player (**d–f** Case #12), and a multi-sport athlete (football, basketball and baseball) (**g–i** Case #21). One case initially without

clinical documentation of contact sports and CTE pathology was subsequently discovered to be a former high school football player (**j–l** Case #4). Microscopically, CTE foci (*arrows and boxes c, f, i, l*) were characterized by clustered glial and neuronal lesions primarily at the depths of sulci and surrounding vessels. *Bar* 2 mm on large sections, 20 μ m on *insets*

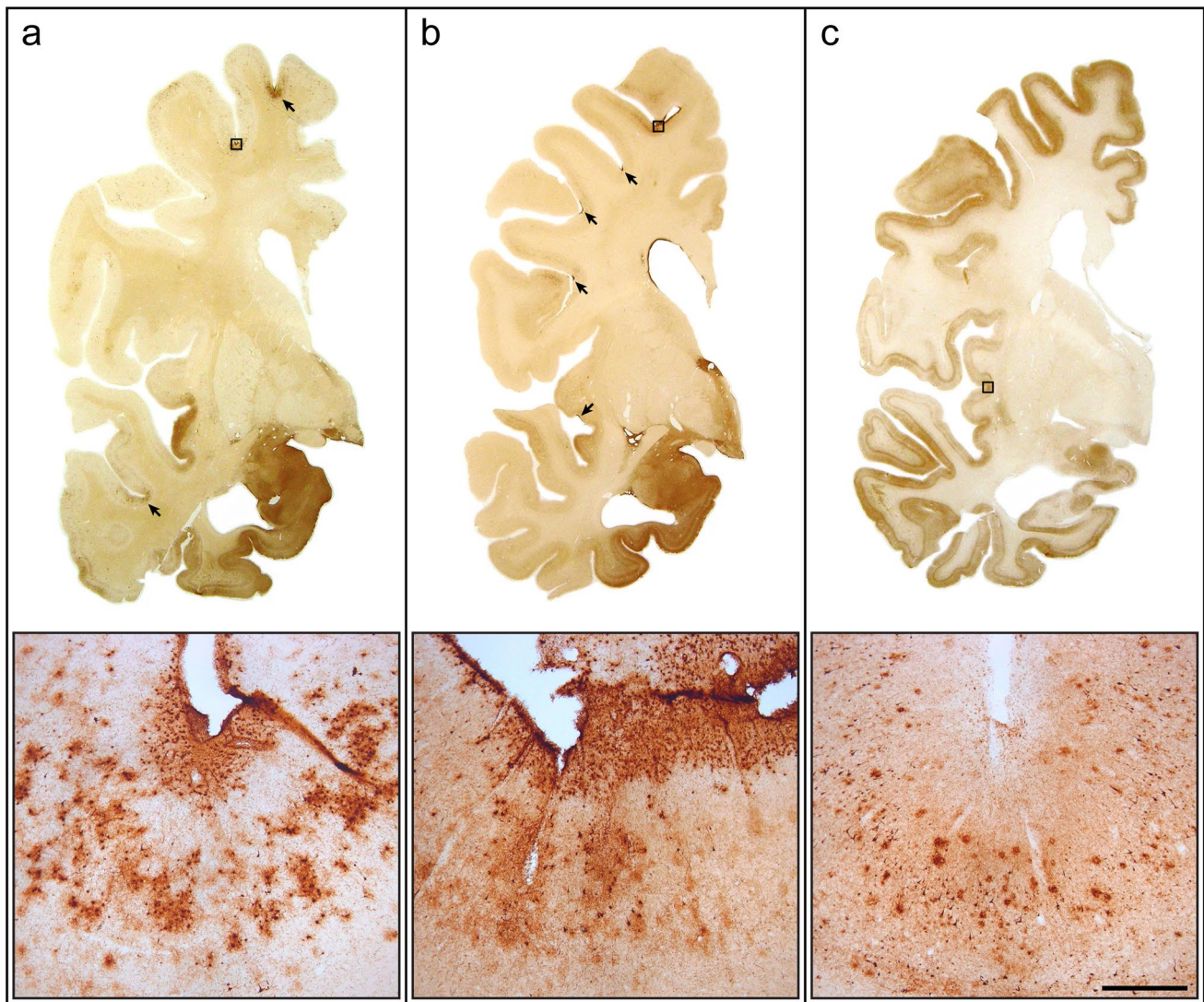


Fig. 3 Distribution of chronic traumatic encephalopathy foci in mixed neurodegenerative cases. In 3 former football players (ages 78, 79 and 81), cortical foci of CTE pathology are observed in the frontal and temporal cortices of two individuals, one with pathologic Alzheimer's disease and Lewy body disease (**a** Case #16) and one with pathologic Lewy body disease (**b** Case #14), but not in a third

individual with pathologic Alzheimer's disease (**c** Case #46). Distributed prominently at the depths of sulci, some foci are large and span the entirety of the cortical gray matter (**a**) while others lesions are discrete, concentrated at the subpial surface (**b**). Bar 15 mm coronal images, 200 μ m inset

classified as CTE Stage I, 7 CTE Stage II, 5 CTE Stage III, and 2 CTE Stage IV. Tau immunohistochemistry was also performed on frontal and parietal cortical tissue of 198 disease- and age-matched controls. No CTE pathology was observed in any control case, including 33 controls with documented head trauma (unrelated to contact sports).

Of the 21 patients with exposure to contact sports and CTE pathology, 11 played football, 5 multiple sports [football plus: baseball (1), basketball and baseball (2), rugby (1), and wrestling (1)], 2 boxing, 1 baseball, 1 basketball, and 1 unspecified involvement with loss of consciousness. Of the 43 patients with exposure to contact sports through

football, 16 (37 %) had CTE pathology, and of these, 6 played up to high school level, 7 played to college, and 1 played professional football. In 27 former football players without evidence of CTE pathology, 15 played up to the high school, 7 played up to college, and none played professional football. There were no significant differences in the proportion of football players with CTE pathology with respect to level of involvement ($P = 0.175$).

The previously defined neurodegenerative conditions in 20 of the 21 patients with CTE pathology included AD ($N = 6$), mixed AD and Lewy body disease (LBD; $N = 4$) (Fig. 3a), pure LBD ($N = 3$) (Fig. 3b), ALS ($N = 3$), and

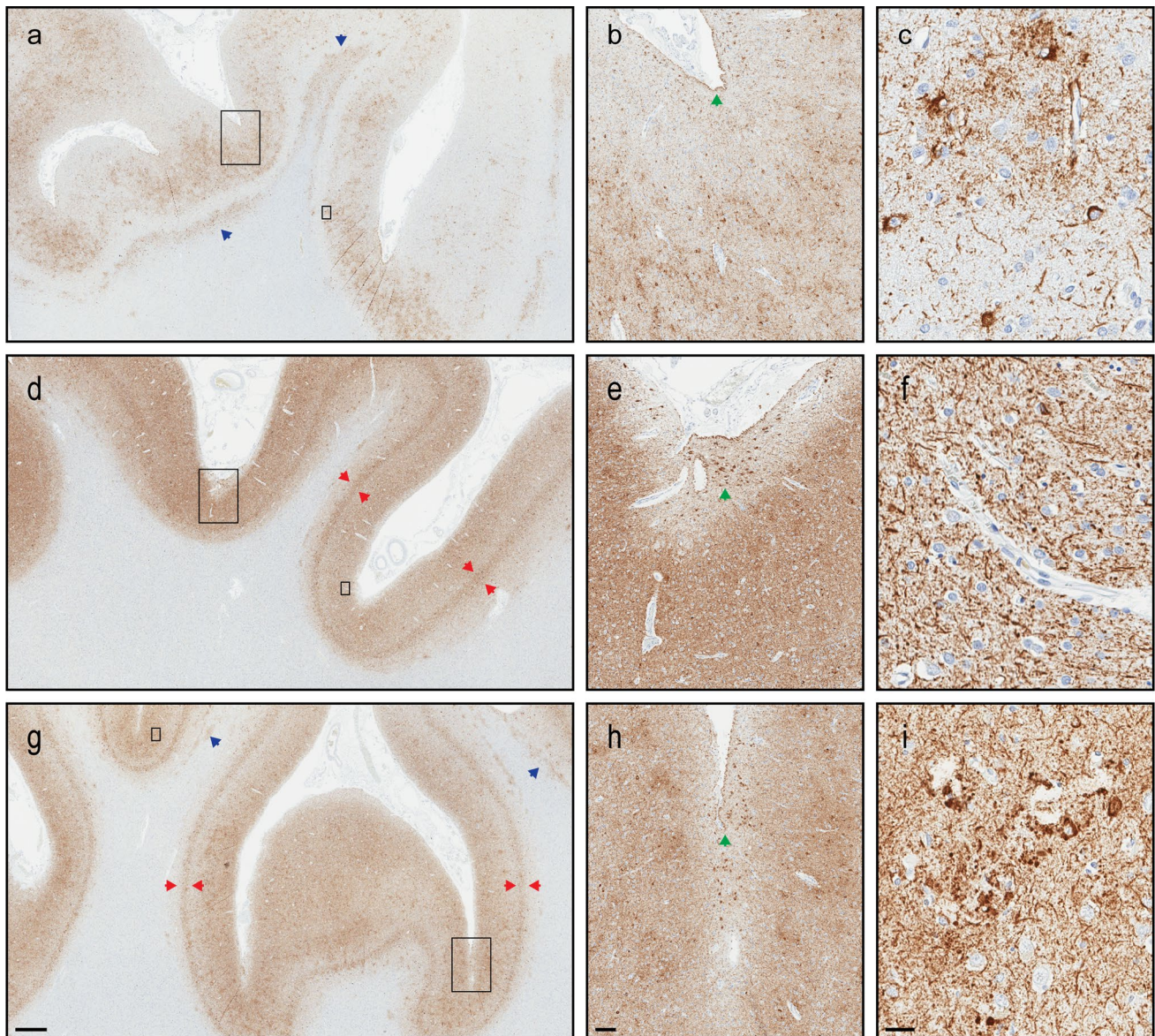


Fig. 4 Neuropathologic differentiation of chronic traumatic encephalopathy and Alzheimer's disease. Tau pathology in the frontal cortex of 3 male amateur boxers, 1 with CTE (Case #11: **a–c**) and 2 without CTE (Case #29: **d–f**; Case #32: **g–i**). All 3 cases have concomitant Alzheimer's disease (Braak Stage 6, Thal Phase 5). Comorbid CTE pathology is patchy (**a**) with marked prominence at the depth of cerebral sulci (**b**). CTE pathognomonic lesions include focal neurofibrillary tangles and astrocytic tau pathology adjacent to penetrating blood vessels (**c**). This contrasts the more uniform laminar distribution of Alzheimer tau pathology (**d**, **g**), particularly in layer 5 (**red arrows**). Similar to Case #11, Case #29 and #32 both show subpial

thorn-shaped astrocytes (**b**, **e**, **h**; **green arrows**) at the depths of sulci. However, this often associated CTE feature is non-specific without additional perivascular pathology (**f**) or neuritic tau pathology associated with cerebral amyloid angiopathy (**i**). Tau-immunoreactive white matter astrocytes (**blue arrows**) are another pathologic feature commonly observed in CTE (**a**) which lack disease specificity (**g**). Ultimately, confluent tau pathology in cases with a high burden of Alzheimer pathology may prohibit a definitive CTE diagnosis. *Large inset b*, **e**, **h**; *small inset: c*, **f**, **i**. Measure bar 1 mm **a**, **d**, **g**; 100 μ m **b**, **e**, **h**; 25 μ m **c**, **f**, **i**

frontotemporal lobar degeneration (FTLD) with fused in sarcoma protein ($N = 1$) or atypical tau protein ($N = 3$). Hippocampal TDP-43 pathology was observed at a similar frequency in former athletes with (7/21; 33 %) and without (14/45; 31 %) CTE pathology (Supplemental Table 1). Comparing the patients with exposure to contact

sports and CTE pathology to those without CTE pathology, there were no significant differences in age at symptom onset, symptom duration, age at death, height, education, history of alcohol or tobacco use, or prior military service (Table 2). There was no increase in frequency of family history of dementia, parkinsonism, or ALS in

Table 2 Comparison of clinical features between former athletes with and without chronic traumatic encephalopathy

Clinical feature (median value)	CTE (#)	Non-CTE (#)	<i>P</i> value
Age at symptom onset (age)	65 (17)	67 (43)	0.646
Symptom duration (number of years)	8 (17)	8 (43)	0.941
Age at death (age)	78 (21)	74 (45)	0.736
Height (cm)	175 (10)	175 (25)	0.686
Education (years)	16 (18)	16 (38)	0.146
History of alcohol use	13Y 5 N	30Y 11 N	1.000
None to rare (<i>no, minimal, periodically</i>)	7	20	–
Low to moderate (<2 drinks/day)	10	14	–
High (≥2 drinks/day; <i>heavy</i>)	1	7	–
History of tobacco use	10Y 8 N	23Y 17 N	0.882
None to rare (<i>no, 0–10 pack years</i>)	10	20	–
Low to moderate (11–30 pack years)	7	10	–
High (30 + pack years)	1	10	–
Military service	12Y 9 N	23Y 22 N	0.847
Clinically documented combat	3	3	–
Family history of neurodegeneration	11Y 10 N	19Y 25 N	0.667

CTE chronic traumatic encephalopathy, N no, Y yes, *italicized text* comment in clinical notes, # number of cases

patients with CTE pathology compared to those without CTE pathology.

Genetic variants associated with neurodegeneration, including *MAPT*, *APOE* and *TMEM106B*, were assessed. Given the small sample size of CTE cases ($N = 17$ with frozen tissue), these results were considered exploratory, and no statistically significant differences were observed (Table 3; Supplemental Table 1). *APOE* $\epsilon 4$ allele tended to be higher in those with exposure to contact sports with (26.5 %) or without (38.4 %) CTE pathology compared to clinical controls (13.4 %). There was a slight increase in the frequency of *MAPT* H1/H1 genotype in men with exposure to contact sports and CTE pathology (70.6 %) compared to those without CTE pathology (62.8 %) and to clinical controls (55.1 %). The H1 haplotype frequency was similar between former athletes with (82.4 %) and without (79.1 %) CTE pathology, both higher than clinical controls (74.4 %). *MAPT* subhaplotyping results are displayed in Supplemental Table 2. There was a decreased frequency of *TMEM106B* homozygous minor allele in patients with exposure to contact sports and CTE pathology (5.9 %) compared to those without CTE pathology (23.3 %) and to clinical controls (17.2 %).

Discussion

Repetitive brain injury sustained during participation in contact sports is considered one of the etiologies of CTE pathology [21]. Contact sports in which CTE pathology has been reported include American football [26, 27], baseball [21], boxing [4], ice hockey [23], rugby [10], soccer [21], and wrestling [28] while other athletic activities including basketball, lacrosse, and martial arts also pose a threat of possible brain injury. Screening for history of exposure to these sports in 1721 men in the Mayo Clinic brain bank for neurodegenerative disorders identified 66 individuals with exposure to contact sports. Football was the most common ($N = 43$), followed by baseball ($N = 10$), boxing ($N = 9$), and basketball ($N = 5$). Of the 66 individuals, 23 played one or more sports during high school, 14 played one or more sports during college, and 15 played their sport beyond college at the amateur ($N = 7$), semi-professional ($N = 5$), or professional level ($N = 3$).

Of the 66 individuals with exposure to contact sports, 21 had pathology consistent with CTE. All had the pathognomonic lesions of CTE pathology [17], namely foci of cortical tau pathology at the depths of sulci and characterized by tau immunoreactive TSA in subpial and perivascular spaces, as well as perivascular NFT and threads (Fig. 2). Two-thirds of the cases displayed mild to moderate CTE pathology (CTE Stages I and II), whereas one-third of the cases harbored more severe CTE pathology (CTE Stages III and IV) [23]. Tau immunohistochemistry demonstrated greater sensitivity to the pathognomonic lesions of CTE than thioflavin S. This finding is reminiscent of thiazin red labeling of other primary tauopathies reported by Uchiyama and colleagues [36]. With 153 unique cases of CTE in the literature as of August 2013 [19], addition of our 21 cases increases this reported frequency by 14 %.

While some of the cases had concomitant Alzheimer-type pathological change, it could be distinguished from CTE pathology (Fig. 4). Whole mount sections were useful to adjudicate difficult cases as the focal nature of epicenters could be more easily appreciated (Fig. 3). It is remotely possible that high Alzheimer-type pathology (Supplemental Table 1) masked subtle CTE pathology. If this is the case, then our results are a conservative estimate of the frequency of CTE pathology. Research from Stein and colleagues has also noted differences in the accumulation of A β plaques in AD and CTE, but suggests select, focal A β pathology might be accelerated in CTE cases [32]. The role of A β pathology in CTE has been and continues to be debated and warrants further research emphasis.

Cortical lesions were most specific for CTE, but underlying white matter astrocytic pathology was also frequent. In this series, white matter astrocytic tau pathology was most marked in two boxers. Tau-immunoreactive

Table 3 *APOE*, *MAPT*, and *TMEM106B* genotypes in men with exposure to contact sports

Variant/genotype	CTE pathology (N = 17)	No CTE pathology (N = 43)	Clinical controls (N = 408)
<i>APOE</i>			
ε2/ε2	0 (0.0 %)	0 (0.0 %)	1 (0.2 %)
ε2/ε3	1 (5.9 %)	2 (4.7 %)	60 (14.7 %)
ε2/ε4	0 (0.0 %)	2 (4.7 %)	7 (1.7 %)
ε3/ε3	8 (47.1 %)	15 (34.9 %)	245 (60.0 %)
ε3/ε4	7 (41.2 %)	17 (39.5 %)	88 (21.6 %)
ε4/ε4	1 (5.9 %)	7 (16.3 %)	7 (1.7 %)
ε2	1 (2.9 %)	4 (4.7 %)	69 (8.5 %)
ε3	24 (70.6 %)	49 (57.0 %)	638 (78.2 %)
ε4	9 (26.5 %)	33 (38.4 %)	109 (13.4 %)
<i>MAPT</i>			
H1/H1	12 (70.6 %)	27 (62.8 %)	225 (55.1 %)
H1/H2	4 (23.5 %)	14 (32.6 %)	157 (38.5 %)
H2/H2	1 (5.9 %)	2 (4.7 %)	26 (6.4 %)
H1	28 (82.4 %)	68 (79.1 %)	607 (74.4 %)
H2	6 (17.6 %)	18 (20.9 %)	209 (25.6 %)
<i>TMEM106B</i> rs3173615			
CC	7 (41.2 %)	7 (16.3 %)	137 (33.6 %)
CG	9 (52.9 %)	26 (60.5 %)	201 (49.3 %)
GG	1 (5.9 %)	10 (23.3 %)	70 (17.2 %)
C	23 (67.6 %)	40 (46.5 %)	475 (58.2 %)
G	11 (32.4 %)	46 (53.5 %)	341 (41.8 %)

astrogliopathy, including subependymal, subpial, gray matter, and white matter astrocytes, is an increasingly recognized neuropathology in elderly brains [18] and not restrictive to CTE. Astrocytic pathology in CTE is often focal and at the depths of sulci, whereas tau astrogliopathy in the elderly can be widespread and has no known predilection for sulcal depths. However, tau astrogliopathy must be considered in the differential diagnosis of CTE, especially those with advanced age.

No statistically significant differences were found between men with exposure to contact sports with CTE pathology and those without CTE pathology for any clinical or demographic feature, including age at symptom onset, symptom duration, age at death, height, and education. Some studies have noted substance abuse is frequent in CTE [23, 25], however, this relationship was not observed in our cohort. Moderate to high alcohol and tobacco usage did not appear to be more prevalent amongst CTE cases compared to former athletes without CTE pathology. CTE pathology has been described in military veterans who have sustained blast injuries [11]. In our cohort of 35 patients who served in the US Armed Forces (6 in a combat role), CTE pathology was not more frequent in veteran athletes than non-veteran athletes.

The genetic underpinnings of CTE have yet to be elucidated. Genetic research conducted to date has focused on *APOE*, a well-known risk factor in AD. While several

studies with smaller cohorts have described a higher frequency of the *APOE* ε4 allele and ε4/ε4 genotype in CTE [13, 20, 33], larger studies have failed to replicate this finding [19, 23]. In the present study, no association was found for *APOE* ε4 and CTE pathology. The significance of the increased frequency of *APOE* ε4 allele observed in those exposed to contact sports, regardless of presence of CTE pathology, is likely coincidental. Since *APOE* is a driving factor for amyloid pathology [14] and CTE is primarily a tauopathy, it was of interest to know whether variants in *MAPT* would be associated with CTE pathology, as has been shown for progressive supranuclear palsy [29], corticobasal degeneration [12] and tangle-predominant dementia [30]. No significant differences were found regarding the frequency of the H1 haplotype, although it did appear higher in the CTE cases compared to controls. It is estimated that more than 50 % of CTE cases have concomitant TDP-43 pathology [22]. The strongest genetic risk factor currently known for TDP-43 pathology in a range of disorders, including FTLN [37], AD [24, 38], and LBD [1], is a common variant in the gene for an uncharacterized lysosomal transmembrane protein 106b (*TMEM106B*). We observed a trend for decrease in homozygous carriers of the protective minor allele of rs3173615 in individuals with exposure to contact sports (Table 3).

In summary, CTE pathology was found in at least 1.2 % of men in a neurodegenerative brain bank exclusive of

progressive supranuclear palsy, corticobasal degeneration, Pick's disease, or a known *MAPT* mutation; however, the frequency was much higher (32 %) in patients with documented participation in contact sports. The results of our screen suggest that individuals with documented participation in contact sports are at an increased risk for CTE pathology compared to those with no such exposure or for whom exposure to contact sports is unknown. The findings have important implications for public health given the number of individuals with past exposure to contact sports. While our brain bank cohort is predominantly Caucasian and enriched in individuals residing in Southeastern United States, the generalizability of our findings is probably greater than that from series of selectively recruited professional athletes [21]. Assessment of frequency or severity of head trauma in affected individuals and the possible clinical correlates of CTE pathology could not be evaluated given the retrospective nature of this study. Our findings should be considered provisional, and it is imperative to screen other brain bank cohorts for CTE pathology, particularly cohorts with minimal concomitant neurodegenerative pathology that can make recognition of the pathognomonic lesion problematic, to determine the frequency of CTE pathology in those with exposure to contact sports. Large cohorts also need to be studied to confirm potential genetic risk for CTE pathology due to *MAPT* and *TMEM106B*, including cohorts prospectively following individuals who do and do not participate in contact sports.

Acknowledgments We would like to thank the patients and their families. Without their generous donation, these studies would not be possible. We would also like to thank Monica Castanedes-Casey, Virginia Philips, and Linda Rousseau for their neurohistologic assistance, Michael DeTure for brain banking, and Deann Gibson for coordination of brain donations. This study was supported by the National Institute of Health (NIH) P50 NS072187 (OAR, ZKW, RR, DWD), R01 NS076471 (RR), R01 NS078086 (OAR), UO1 NS086659-01 (ACM), P30 AG13846 (ACM; supplement 0572063345-5). Other support included an unrestricted gift from Carl Edward Bolch Jr. and Susan Bass Bolch (ZKW), Department of Veterans Affairs (ACM), Veterans Affairs Biorepository CSP 501 (ACM), Sports Legacy Institute (ACM), National Operating Committee on Standards for Athletic Equipment (ACM), the National Football League (ACM), the Andlinger Foundation (ACM), and the WWE (ACM).

References

- Aoki N, Murray ME, Ogaki K et al (2015) Hippocampal sclerosis in Lewy body disease is a TDP-43 proteinopathy similar to FTLTDP Type A. *Acta Neuropathol* 129:53–64
- Braak H, Braak E (1991) Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 82:239–259
- Cantu RC, Mueller FO (2009) The prevention of catastrophic head and spine injuries in high school and college sports. *Br J Sports Med* 43:981–986
- Corsellis JA, Bruton CJ, Freeman-Browne D (1973) The aftermath of boxing. *Psychol Med* 3:270–303
- DeKosky ST, Blennow K, Ikonovic MD, Gandy S (2013) Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. *Nat Rev Neurol* 9:192–200
- DeKosky ST, Ikonovic MD, Gandy S (2010) Traumatic brain injury—football, warfare, and long-term effects. *N Engl J Med* 363:1293–1296
- Fournier CN, Gearing M, Upadhyayula SR, Klein M, Glass JD (2015) Head injury does not alter disease progression or neuropathologic outcomes in ALS. *Neurology* 84:1788–1795
- Gavett BE, Stern RA, Cantu RC, Nowinski CJ, McKee AC (2010) Mild traumatic brain injury: a risk factor for neurodegeneration. *Alzheimers Res Ther* 2:18
- Gavett BE, Stern RA, McKee AC (2011) Chronic traumatic encephalopathy: a potential late effect of sport-related concussive and subconcussive head trauma. *Clin Sports Med* 30(1):179–188
- Geddes JF, Vowles GH, Nicoll JA, Revesz T (1999) Neuronal cytoskeletal changes are an early consequence of repetitive head injury. *Acta Neuropathol* 98:171–178
- Goldstein LE, Fisher AM, Tagge CA et al (2012) Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Sci Transl Med* 4:134ra160
- Houlden H, Baker M, Morris HR et al (2001) Corticobasal degeneration and progressive supranuclear palsy share a common tau haplotype. *Neurology* 56:1702–1706
- Jordan BD, Kanik AB, Horwich MS et al (1995) Apolipoprotein E epsilon 4 and fatal cerebral amyloid angiopathy associated with dementia pugilistica. *Ann Neurol* 38:698–699
- Josephs KA, Tsuboi Y, Cookson N, Watt H, Dickson DW (2004) Apolipoprotein E epsilon 4 is a determinant for Alzheimer-type pathologic features in tauopathies, synucleinopathies, and frontotemporal degeneration. *Arch Neurol* 61:1579–1584
- King A, Sweeney F, Bodi I et al (2010) Abnormal TDP-43 expression is identified in the neocortex in cases of dementia pugilistica, but is mainly confined to the limbic system when identified in high and moderate stages of Alzheimer's disease. *Neuropathology* 30:408–419
- Kokjohn TA, Maarouf CL, Dausgs ID et al (2013) Neurochemical profile of dementia pugilistica. *J Neurotrauma* 30:981–997
- Koroshetz W (2015) Report from the First NIH consensus conference to define the neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. <http://www.ninds.nih.gov/research/tbi/ReportFirstNIHConsensusConference.htm>
- Kovacs GG, Milenkovic I, Wohrer A et al (2013) Non-Alzheimer neurodegenerative pathologies and their combinations are more frequent than commonly believed in the elderly brain: a community-based autopsy series. *Acta Neuropathol* 126:365–384
- Maroon JC, Winkelman R, Bost J et al (2015) Chronic traumatic encephalopathy in contact sports: a systematic review of all reported pathological cases. *PLoS One* 10(2):e0117338
- McKee AC, Cantu RC, Nowinski CJ et al (2009) Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol* 68:709–735
- McKee AC, Daneshvar DH, Alvarez VE, Stein TD (2014) The neuropathology of sport. *Acta Neuropathol* 127:29–51
- McKee AC, Gavett BE, Stern RA et al (2010) TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. *J Neuropathol Exp Neurol* 69:918–929
- McKee AC, Stern RA, Nowinski CJ et al (2013) The spectrum of disease in chronic traumatic encephalopathy. *Brain* 136:43–64
- Murray ME, Cannon A, Graff-Radford NR et al (2014) Differential clinicopathologic and genetic features of late-onset amnesic dementias. *Acta Neuropathol* 128:411–421
- Omalu B, Bailes J, Hamilton RL et al (2011) Emerging histomorphologic phenotypes of chronic traumatic encephalopathy in American athletes. *Neurosurgery* 69:173–183

26. Omalu BI, DeKosky ST, Hamilton RL et al (2006) Chronic traumatic encephalopathy in a national football league player: part II. *Neurosurgery* 59:1086–1092 (**discussion 1092–1083**)
27. Omalu BI, DeKosky ST, Minster RL et al (2005) Chronic traumatic encephalopathy in a National Football League player. *Neurosurgery* 57:128–134 (**discussion 128–134**)
28. Omalu BI, Fitzsimmons RP, Hammers J, Bailes J (2010) Chronic traumatic encephalopathy in a professional American wrestler. *J Forensic Nurs* 6:130–136
29. Rademakers R, Melquist S, Cruts M et al (2005) High-density SNP haplotyping suggests altered regulation of tau gene expression in progressive supranuclear palsy. *Hum Mol Genet* 14:3281–3292
30. Santa-Maria I, Haggiagi A, Liu XM et al (2012) The MAPT H1 haplotype is associated with tangle-predominant dementia. *Acta Neuropathol* 124:693–704
31. Smith DH, Johnson VE, Stewart W (2013) Chronic neuropathologies of single and repetitive TBI: substrates of dementia? *Nat Rev Neurol* 9:211–221
32. Stein TD, Montenegro PH, Alvarez VE et al (2015) Beta-amyloid deposition in chronic traumatic encephalopathy. *Acta Neuropathol* 130:21–34
33. Stern RA, Daneshvar DH, Baugh CM et al (2013) Clinical presentation of chronic traumatic encephalopathy. *Neurology* 81:1122–1129
34. Thal DR, Rub U, Orantes M, Braak H (2002) Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 58:1791–1800
35. Turner RC, Lucke-Wold BP, Robson MJ et al (2012) Repetitive traumatic brain injury and development of chronic traumatic encephalopathy: a potential role for biomarkers in diagnosis, prognosis, and treatment? *Front Neurol* 3:186
36. Uchihara T, Nakamura A, Yamazaki M et al (2001) Different conformation of neuronal tau deposits distinguished by double immunofluorescence with AT8 and thiazin red combined with Gallyas method. *Acta Neuropathol* 102:462–466
37. Van Deerlin VM, Sleiman PMA, Martinez-Lage M et al (2010) Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP43 inclusions. *Nat Genet* 42:U234–U234
38. Yu L, De Jager PL, Yang J et al (2015) The TMEM106B locus and TDP-43 pathology in older persons without FTL. *Neurology* 84:927–934