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ORIGINAL PAPER

Repetitive head impacts and chronic traumatic encephalopathy are associated with TDP-43 inclusions and hippocampal sclerosis

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Abstract

Hippocampal sclerosis (HS) is associated with advanced age as well as transactive response DNA-binding protein with 43 kDa (TDP-43) deposits. Both hippocampal sclerosis and TDP-43 proteinopathy have also been described in chronic traumatic encephalopathy (CTE), a neurodegenerative disease linked to exposure to repetitive head impacts (RHI). However, the prevalence of HS in CTE, the pattern of TDP-43 pathology, and associations of HS and TDP-43 with RHI are unknown. A group of participants with a history of RHI and CTE at autopsy ($n=401$) as well as a group with HS-aging without CTE ($n=33$) was examined to determine the prevalence of HS and TDP-43 inclusions in CTE and to compare the clinical and pathological features of HS and TDP-43 inclusions in CTE to HS-aging. In CTE, HS was present in 23.4%, and TDP-43 inclusions were present in 43.3% of participants. HS in CTE occurred at a relatively young age (mean 77.0 years) and was associated with a greater number of years of RHI than CTE without HS adjusting for age ($p=0.029$). In CTE, TDP-43 inclusions occurred frequently in the frontal cortex and occurred both with and without limbic TDP-43. Additionally, structural equation modeling demonstrated that RHI exposure years were associated with hippocampal TDP-43 inclusions ($p<0.001$) through increased CTE stage ($p<0.001$). Overall, RHI and the development of CTE pathology may contribute to TDP-43 deposition and hippocampal sclerosis.

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Introduction

Hippocampal sclerosis associated with aging (HS-aging and hereafter referred to as HS for simplicity) is a common finding in the older population (10–26% of those older than 80 years old) [3, 52]. HS consists of neuronal loss and gliosis in region CA1 of the hippocampus and/or subiculum that is not attributed to microinfarcts or Alzheimer disease (AD). HS has been associated with transactive response DNA-binding protein with 43 kDa (TDP-43) inclusions in as many as 71–86% of cases [22, 24, 46, 52] and is a common finding in frontotemporal lobar degeneration with TDP-43 (FTLD-TDP). TDP-43 inclusions are present in multiple different diseases, including FTLD, amyotrophic lateral sclerosis (ALS), and limbic-associated TDP-43 encephalopathy neuropathologic change (LATE-NC) [24, 49, 58]. Chronic traumatic encephalopathy (CTE) is an increasingly recognized neurodegenerative disease associated with repetitive head impacts (RHI) that typically occur in contact sports, such as American football, boxing, hockey, rugby, and others. CTE is characterized by tau pathology in a distribution distinct from that seen in other tau-associated pathologies such as AD. In CTE, hyperphosphorylated tau (p-tau) deposition occurs as neurofibrillary tangles, and neurites, with or without astrocytic tangles, in perivascular clusters of the cerebral cortex and is most pronounced at the sulcal depths [36, 37, 39]. CTE is unique from other tau-defined neuropathological diseases such as primary age-related tauopathy (PART) [11] and age-related tau astrogliopathy (ARTAG) [29]. Numerous other pathologies may be influenced by RHI and associated with CTE, including beta-amyloid plaques [57], cerebral amyloid angiopathy [56], motor neuron disease [13, 37, 42, 60], and FTLD. Additionally, Lewy body disease has previously been linked to HS pathology [7] and CTE pathology [1, 5].

TDP-43 inclusions have been described in a subset of typically high-stage CTE. They include intraneuronal cytoplasmic inclusions and neuritic thread-like inclusions [21, 28, 31, 38, 54], which can be present in sulcal depths along with tau deposits [14]. However, the involvement of limbic structures and association with HS is not well understood. Furthermore, although HS has been previously reported in CTE [19, 33, 40, 61], its prevalence in CTE and its association with TDP-43 and RHI are unknown. Here, we set out to determine the prevalence of HS and TDP-43 inclusions in CTE and compare the clinical and pathological features of HS and TDP-43 in CTE to a group of participants with HS without CTE. In addition, we further tested the hypothesis that RHI was associated with HS and TDP-43 in CTE.

Materials and methods

Participants

Autopsy participants with a history of RHI exposure through contact sports participation and neuropathologically diagnosed with CTE ($n = 401$) were drawn from the Understanding Neurologic Injury and Traumatic Encephalopathy (UNITE) study as part of the Veterans Affairs-Boston University-Concussion Legacy Foundation (VA-BU-CLF) brain bank and from the Framingham Heart Study. Inclusion criteria for UNITE included a history of contact sports participation, military service, or domestic violence [43]. One case was not used for analysis due to a history of epilepsy. For a comparison group, we selected brain donors with HS and without CTE from the Boston University Alzheimer's Disease Research Center (BUADRC, mean age: 83 years), Framingham Heart Study (mean age: 87 years), Massachusetts Alzheimer's Disease Research Center (MADRC, mean age: 80 years), Charlestown, MA, and Massachusetts General Hospital (MGH), Boston, MA. Consent for the use of tissue for research purposes was obtained from the legal healthcare proxy by the respective institutions. IRB approval was granted by Boston University Medical Center (BUMC) and the Bedford VA Healthcare System, MA.

Neuropathological assessment

Neuropathological processing included comprehensive screening for neurodegenerative conditions following procedures established for the VA-BU-CLF brain bank [43, 59]. Briefly, all cases were evaluated based on paraffin-embedded tissue sections taken from standardized regions for histochemical and immunohistochemical staining. CTE was neuropathologically diagnosed using the NIH consensus criteria, which include abnormal perivascular accumulations of hyperphosphorylated tau (p-tau) in neurons, with or without astrocytes, and cell processes concentrated at the sulcal depths [36]. CTE was then staged based on regional p-tau involvement [2, 36]. Neuronal p-tau was required for diagnosis and to distinguish from aging-related tau astrogliopathy. A diagnosis of HS was determined by the presence of severe neuronal loss and gliosis without significant ghost tangles within subiculum/CA1 and variably involving other hippocampal neuronal fields in the absence of other pathologic findings that could account for neuronal loss in this region [16, 32, 52].

For cases needing additional TDP-43 immunohistochemistry, paraffin-embedded material was retrieved from storage from the BUADC, MGH, and MADRC. All tissues

were previously fixed in formalin or paraformaldehyde. Tissues from BUADC were processed in a research laboratory on a Tissue-Tek VIP 2000 processor (Miles Laboratories, Inc., Naperville, IL). Tissues from the MADRC were treated with 88% formic acid for 60 min after formalin fixation and processed in the research lab on a Thermo Scientific Excelsior ES tissue processor (Thermo Fisher Scientific, Waltham, MA, USA). Sections were cut at 7–10 µm from the following three blocks when available: superior frontal (Brodmann area [BA] 8,9), hippocampus at the level of the lateral geniculate nucleus (LGN), and amygdala with the entorhinal cortex (BA28). Immunohistochemistry with *p*-TDP-43 (Cosmo Bio, Tokyo, Japan) was performed on all unstained sections at the MARDC research laboratory at a titration of 1:3000 and processed on a Leica Bond RX automated stainer (Leica Biosystems, Wetzlar, Germany) according to the manufacturer's instructions. Positive controls were verified with each batch of stains.

All sections were examined for the presence of *p*-TDP-43 inclusions of all types. As most inclusions were in the form of neuronal cytoplasmic inclusions (NCIs) and dystrophic neurites (DNs), these were scored as none (0), mild (1), moderate (2), or severe (3). The following regions were scored in the hippocampal block: cornu ammonis 1–4 (CA1, CA2, CA3, CA4), subiculum, parahippocampal gyrus, and occipitotemporal gyrus; inferior temporal gyrus was scored when present on the section. The amygdala, entorhinal cortex, and transentorhinal region were scored separately in the amygdala block. Frontal cortex TDP-43 inclusions were evaluated within the dorsolateral frontal cortex. Differences between CTE/HS and the CTE/No HS groups were examined for the following variables: CTE stage (I–IV), presence of dorsolateral frontal neurofibrillary tangles (score 0–3), Thal (0–5), Braak (stage 0–VI), and CERAD (none, sparse, moderate or frequent) and NIA score (not, low, intermediate or high). Comparisons for vascular pathology were also made with the following variables: arteriolosclerosis, atherosclerosis, cerebral amyloid angiopathy, and white matter rarefaction (with each category dichotomized as none/mild or moderate/severe). We also examined for microinfarcts (presence or absence) as well as the presence of other pathologies [frontotemporal lobar degeneration (FTLD), motor neuron disease (MND), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and Pick's disease (PiD)].

Genotyping

DNA was extracted from fresh-frozen brain tissue using the Maxwell RSC Tissue DNA Kit (Promega, Madison, WI) according to the manufacturer's protocol. *Apolipoprotein E (APOE)* genotype was determined using two single-nucleotide polymorphisms (National Center for Biotechnology Information SNPs *rs429358* and *rs7412*). In addition, *transmembrane protein 106B (TMEM106B; rs1990622)* and *granulin precursor (GRN; rs5848*) SNPs were determined using TaqMan SNP genotyping assay on the Step One Plus Real-Time PCR System (Applied Biosystems, Waltham, MA). The CTE samples were genotyped using GWAS microarray chips (Illumina, Inc., San Diego, CA). Standard QC procedures for the genotype data were performed using PLINK [9], which include excluding SNPs with excess missingness, divergence from Hardy–Weinberg Equilibrium ($p < 10–6$), and minor allele frequency (MAF) < 1%, as well as subjects with sex mismatch between clinical and genetic data, low call rate (< 95%) and who were related (i.e., identify-by-descent estimate > 0.2). We imputed the allelic dosage of *ATP-binding cassette transporter sub-family C member 9 (ABCC9*; rs1914361) using TOPMed reference panel (https://imputation.biodatacatalyst.nhlbi.nih.gov) [9, 53].

Statistical analysis

Statistical analyses were performed with SPSS v25.0 (IBM Corp, Armonk, NY) and SAS (SAS Institute Inc, Cary, NC). When comparing all three groups (CTE/No HS, CTE/HS, and HS/No CTE), the means were compared between groups using the Kruskal–Wallis, and values were adjusted by the Bonferroni correction for multiple tests for continuous and ordinal variables. The Chi-square test was used for proportions to evaluate dichotomous variables. Estimated means and standard error (SEM) of the TDP-43 severity score for those with TDP-43 pathology in the given region were calculated using analysis of covariance (ANCOVA) adjusting for age. Comparisons between the CTE/HS group and the CTE/No HS group were made for age at death, race, type of sport, and total years of sport played (Table 1). The effects of putative genetic risk variants in CTE were tested utilizing binary logistic regression with HS as the outcome adjusting for age at death and separately testing dominant, additive, and recessive models. Years of football play was used as a proxy for RHI as this was the primary sport for the majority of participants and has been shown to have a clear association with both CTE status and stage regardless of selection bias [41]. For a final model, we employed structural equation modeling [20] on the total sample of CTE participants with a history of American football play. For this model, HS and hippocampal TDP-43 were the dependent variables, and RHI, age, and CTE stage were predictors to determine the direct and total effects. Statistical significance throughout was set as α of 0.05. Sensitivity analyses stratifying by age at death and age of cognitive symptom onset were also performed.

Table 1 Demographic and exposure characteristics in participants with and without CTE and hippocampal sclerosis

Characteristic	CTE/No HS	CTE/HS	HS/No CTE	*p* value
Sample size (*n*)	307	94	33	
Age, mean (SE)	57.7 (1.1)	77.0 (0.80)	86.6 (1.5)	**< 0.001**†‡*
Age, range	20–91	52–100	69–102	
Race (%)				
African American	54 (17.8)	13 (14.3)	–	0.395
Caucasian	243 (80.2)	76 (83.5)	–	
American native Indian	1 (0.3)	1 (1.1)	–	
Asian	0 (0)	1 (1.1)	–	
Pacific Islander	1 (0.3)	0 (0)	–	
Other	4 (1.4)	0 (0)	–	
Contact sport exposure				
Total years of play, mean (SE)	15.8 (0.4)	17.9 (0.9)	–	**0.029**
Total yeas of football, mean (SE)	13.7 (0.3)	15.1 (0.7)	–	0.073
Main sport played, *n* (%)	296	90		
Football	252 (85.1)	76 (84.4)		0.896
Hockey	18 (6.1)	3 (3.3)		
Boxing	5 (1.7)	8 (8.9)		
Other[a]	21 (7.1)	3 (3.3)		

The Kruskal–Wallis statistical test was performed on continuous variables and Chi-squared test was used for categorical variables. Post hoc pairwise comparisons were adjusted by the Bonferroni correction for multiple tests

$p < 0.05$ in bold. *$p < 0.05$ between CTE/HS and HS/No CTE; †$p < 0.05$ between CTE/HS and CTE/No HS; ‡$p < 0.05$ between HS/No CTE and CTE/No HS

[a]Other sports include rugby, mixed martial arts, lacrosse, pro wrestling, bull riding, and wrestling. CTE: chronic traumatic encephalopathy

Results

Hippocampal sclerosis in CTE

Of the participants with CTE, there were 94 with HS (CTE/HS) and 307 without HS (CTE/No HS), resulting in a frequency of HS in CTE of 23.4%. The mean age of the CTE/HS group (77.0 yrs) was significantly older than the CTE/No HS group (57.7, $p = < 0.001$), which is consistent with the positive association of HS with age. However, the average age of death in the CTE/HS group was significantly younger than in the HS/No CTE group (86.6 years, $p < 0.001$), although this may be biased by the older recruitment age of ADRCs versus UNITE. The age breakdown by decade of CTE/No HS and CTE/HS is listed in the Supplementary Table 1, online resource. The racial composition of the CTE/No HS and CTE/HS groups was similar, and there were no available race data for the HS/No CTE group. Within the CTE groups, there were significantly more years of contact sports play in the CTE/HS group (17.9 years) compared to the CTE/No HS group adjusting for age at death (15.8 years, $p = 0.029$). Both groups were primarily comprised of football players, with much smaller numbers of hockey, boxing, and other sports exposure histories present (Table 1).

As expected, given the large age differences between groups, age-related pathologies were more common in HS groups. Those with HS (with or without CTE) were significantly more likely to have Alzheimer's disease pathology with higher frequencies of CERAD, Thal, and NIA neuropathological AD change scores (p's < 0.05, Table 2). Those with HS (CTE or not) were significantly more likely to have a moderate/severe burden of arteriolosclerosis and atherosclerosis than the CTE/No HS group (Table 2). Similarly, the HS groups had higher burdens of cerebral amyloid angiopathy and white matter rarefaction (Table 2). Those with sclerosis were also more likely to have microinfarcts. Significant differences were also seen for FTLD, which had a higher prevalence in the CTE/HS group ($p < 0.001$, Table 2). Most of the TDP-43 inclusions were intraneuronal cytoplasmic and short dystrophic neurites in the superficial layers consistent with FTLD, type A. Additionally, Lewy body pathology was much higher in the CTE/HS group ($p = 0.03$) when compared to CTE/ No HS, and HS/ No CTE ($p < 0.001$). A secondary analysis matching the age range of the CTE/HS group and excluding those < 52 years at death, showed that the pathological findings from Tables 1 and 2 still retained significance, including more years of contact sports play in CTE/HS compared to CTE/No HS ($p = 0.012$).

Table 2 Neuropathological characteristics of participants with and without CTE and hippocampal sclerosis

Characteristic	CTE/No HS	CTE/HS	HS/No CTE	*p* value
TDP-43 inclusions, *n* (%)	87 (30.5)	90 (95.7)	33 (100.0)	**< 0.001**†‡
Amygdala	14 (4.9)	13 (13.8)	0 (0)	**0.003***†
Hippocampus	29 (10.2)	38 (40.4)	27 (81.8)	**< 0.001***,†,‡
Frontal + limbic[a]	31 (10.9)	39 (41.5)	6 (18.2)	**< 0.001***†
Frontal only	13 (5.7)	0 (0.0)	0 (0)	**< 0.001**†,‡
CTE Stage, *n* (%)				
Stage I	60 (19.5)	4 (4.3)	–	**< 0.001**
Stage II	77 (25.1)	7 (7.4)	–	
Stage III	122 (39.7)	19 (20.2)	–	
Stage IV	48 (15.6)	64 (68.1)	–	
DLF NFT (Tau)				
Positive (%)	279 (91.5)	91 (96.8)	–	0.575
Thal phase, *n* (%)				
0 (A0)	167 (54.6)	14 (15.1)	–	**< 0.001**
1 (A1)	32 (10.5)	12 (12.9)	–	
2–3 (A2)	41 (13.4)	20 (21.5)	–	
4–5 (A3)	66 (21.6)	47 (50.5)	–	
Braak stage, *n* (%)				
0 (B0)	77 (25.1)	7 (7.7)	2 (6.5)	**< 0.001**†‡
I–II (B1)	78 (25.4)	15 (16.5)	6 (19.4)	
III–IV (B2)	112 (36.5)	42 (46.2)	6 (19.4)	
V–VI (B3)	40 (13.0)	27 (29.7)	17 (54.8)	
CERAD, *n* (%)				
Absent (C0)	223 (73.0)	33 (35.1)	9 (28.1)	**< 0.001**†‡
Sparse (C1)	50 (16.3)	43 (45.7)	4 (12.5)	
Moderate (C2)	22 (7.2)	12 (12.8)	9 (28.1)	
Frequent (C3)	11 (3.6)	6 (6.4)	10 (31.3)	
Alzheimer disease *n* (%)				
Not	196 (63.8)	29 (30.9)	4 (15.4)	**< 0.001***†‡
Low	36 (11.7)	15 (16.0)	2 (7.7)	
Intermediate	49 (16.0)	34 (36.2)	9 (34.6)	
High	26 (8.5)	16 (17.0)	11 (42.3)	
Vascular pathology				
Arteriolosclerosis, *n* (%)				
None/mild	181 (59.3)	29 (31.2)	7 (22.6)	0.651
Moderate/severe	124 (40.7)	64 (68.8)	24 (77.4)	
Atherosclerosis, *n* (%)				
None/mild	245 (86.0)	59 (64.1)	4 (44.4)	0.090
Moderate/severe	40 (14.0)	33 (35.9)	5 (55.6)	
Cerebral amyloid angiopathy, *n* (%)				
None/mild	265 (87.2)	65 (69.1)	23 (71.9)	0.282
Moderate/severe	39 (12.8)	29 (30.9)	9 (28.1)	
WM rarefaction, *n* (%)				
None/mild	174 (57.6)	36 (39.1)	–	0.714
Moderate/severe	128 (42.4)	56 (60.9)	–	
Microinfarcts, *n* (%)				
No	248 (80.8)	60 (63.8)	–	
Yes	59 (19.2)	34 (36.2)	–	0.847
Other pathologies				
FTLD-TDP	3 (1.0)	22 (23.4)	4 (12.5)	**< 0.001**†‡
MND	17(5.5)	4 (4.3)	1 (3.2)	0.771

Table 2 (continued)

Characteristic	CTE/No HS	CTE/HS	HS/No CTE	*p* value
Argyrophilic grain disease	7(2.3)	2(2.1)	0 (0)	0.930
PSP	3 (1.0)	3 (3.2)	4 (12.9)	**< 0.001***‡
CBD	1 (0.3)	0 (0)	0 (0)	0.580
PiD	0 (0)	1 (1.1)	0 (0)	0.526
Lewy body pathology	25 (8.2)	18 (19.1)	1 (3.1)	**0.003***†

The Kruskal–Wallis statistical test was performed on continuous variables and Chi-squared test was used for categorical variables. Post hoc pairwise comparisons were adjusted by the Bonferroni correction for multiple tests

$p < 0.05$ in bold. *$p < 0.05$ between CTE/HS and HS/No CTE; †$p < 0.05$ between CTE/HS and CTE/No HS; ‡$p < 0.05$ between HS/No CTE and CTE/No HS

[a]Includes cases that have amygdala and frontal cortex TPD-43 inclusions without hippocampal TDP-43 inclusions ($n = 1$ for CTE/No HS, n = 4 for CTE/HS)

CTE chronic traumatic encephalopathy, *HS* hippocampal sclerosis, *PSP* progressive supranuclear palsy, *CBD* corticobasal degeneration, *PiD* Pick's disease, *MND* motor neuron disease, *FTLD-TDP* frontotemporal lobar degeneration with TDP-43

TDP-43 pathology in CTE

Overall, TDP-43 inclusions were present in 43.3% of participants with CTE. Of those CTE participants with TDP-43, most TDP-43 inclusions were present in limbic regions only (53%) followed by limbic plus frontal regions (40%) with a subset involving frontal cortex only (7%). Within the CTE/HS group, 95.7% showed limbic TDP-43, suggesting that in a few participants ($n = 4$), pathology other than TDP-43 underlies hippocampal sclerosis (Table 2). Of these four cases with hippocampal sclerosis and no TDP-43 inclusions, two can be explained by severe tau deposits in the hippocampus. Strikingly, the number of cases with CTE with limbic and frontal TDP-43 was much higher in the CTE/HS group (41.5%) than in the HS/No CTE group (18.2%) despite the CTE/HS group having a significantly lower age at death. Within CTE, but not HS alone, there were 6 cases that did have TDP-43 pathology in the amygdala and frontal cortex but not within the hippocampus ($n = 1$ for CTE/No HS, $n = 5$ for CTE/HS; Table 2).

Frontal cortex TDP-43 inclusions have been previously reported in participants with CTE. Indeed, of those with CTE, there were 13 (7%) with TDP-43 inclusions in the frontal cortex, but not in the amygdala, hippocampus, or other medial temporal lobe structures (Table 2), suggesting a pattern of TDP-43 deposition that is more common in CTE and distinguishable from LATE-NC. Additionally, temporal cortex TDP-43 pathology was present in three cases (1.3%) without TDP-43 inclusions in the frontal cortex, amygdala, or hippocampus.

When present, the types of TDP-43 inclusions were similar between groups and included NCIs and/or DNs. Differences between severity were scored as none, mild, moderate, and severe. An example of scoring in the dentate gyrus of the hippocampus is provided (Fig. 1). In most instances, the severity scores were similar for NCIs and DNs in the various hippocampal subregions when present (Fig. 2a and b). This pattern of roughly equivalent numbers of NCIs and short DNs would best fit into the class A pattern (Mackenzie type 1 or Sampathu type 3) classification system described for TDP-43 staining in FTLD and also described in HS [34,

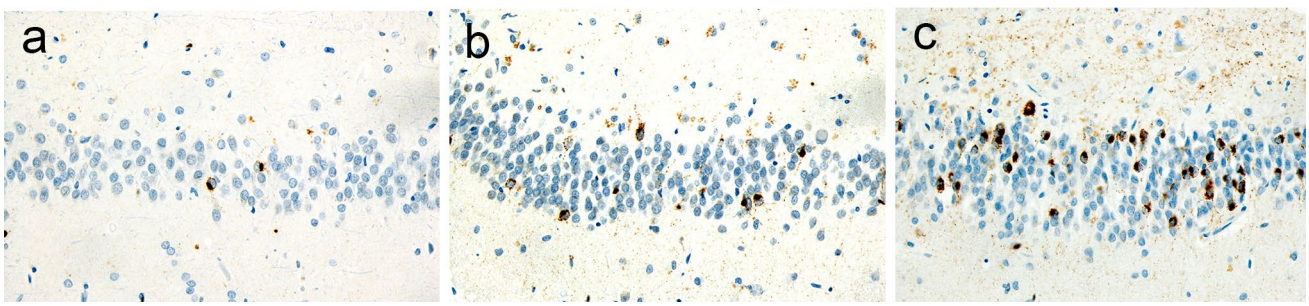

Fig. 1 Immunohistochemistry for TDP-43 in the dentate gyrus of the hippocampus at the level of the lateral geniculate nucleus from CTE/HS cases. Examples of mild (**a**), moderate (**b**), and severe (**c**)

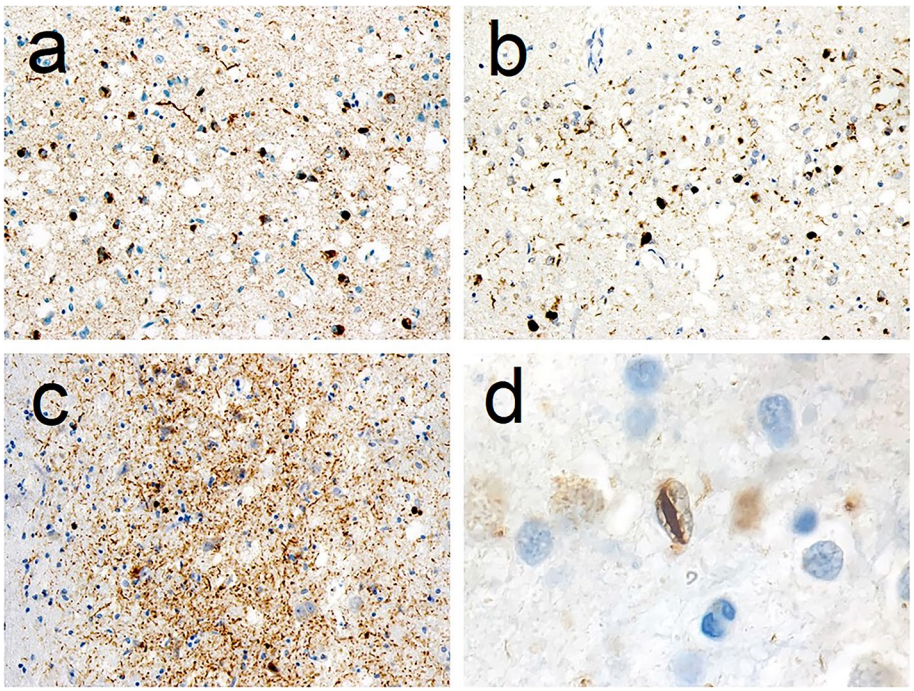

Fig. 2 Immunohistochemistry for TDP-43 in cases of CTE/HS demonstrates primarily a mixture of neuronal cytoplasmic inclusions and short dystrophic neurites (**a**, **b**). Less commonly, the dystrophic neurites predominated (**c**). This was seen in regions with severe or complete pyramidal dropout. Rarely, lentiform intranuclear inclusions could also be seen (**d**)

35, 55]. Patterns of elongated DNs were not appreciated. In some cases of severe hippocampal sclerosis with large regions of complete pyramidal loss within CA1 or the subiculum, short DNs predominated, but this was likely due to the complete absence of neurons in these regions (Fig. 2c). Abnormal lentiform intraneuronal inclusions could also be seen, but these were exceptionally rare (Fig. 2d). In a few CTE cases, the pattern of TDP-43 deposition in the dorsolateral frontal cortex mimicked the pattern of tau deposition seen in CTE. TDP-43 pathology was isolated at the sulcal depths (Fig. 3a) and in a perivascular distribution with a mix of glial and neuronal inclusions (Fig. 3b and c). In both groups with HS, TDP-43 was present within multiple hippocampal subregions (Table 3).

Hippocampal TDP-43 burden in CTE

We examined the distribution and severity of TDP-43 inclusions within hippocampal subfields between CTE-HS and HS alone in those participants with TDP-43 (Table 3). The CA1 subfield was less likely to have TDP-43 inclusions ($p = 0.003$), and when present, the TDP-43 severity score was lower ($p = 0.012$) in CTE/HS than HS/No CTE. On the other hand, the CA2 subfield had a similar frequency of TDP-43 involvement but a greater severity score in CTE/HS than HS alone ($p = 0.044$). In addition, the dentate gyrus was less frequently involved ($p = 0.002$), but when present TDP-43 severity was worse in CTE/HS ($p = 0.015$) (Table 3). Figure 4 illustrates the differences in TDP-43 severity scores between CTE/HS and HS alone within hippocampal subfields. Positive differences are shown in shades of red and illustrate worse severity scores in CTE/HS (in CA2, CA4 and dentate gyrus), and negative differences are shown in shades of blue and represent lower TDP-43 severity scores in CTE/HS (in CA1 and subiculum) compared to HS alone. Each bar of color on the gradient bar represents a 0.1 change between mean scores (Table 3).

Genetic risk factors

The major known risk factors linked to hippocampal sclerosis and LATE-NC are genetic variations in *TMEM106B*, *GRN*, *APOE*, and *ABCC9* [15, 17, 27, 45, 49, 50]. None of these genes showed a significant association between risk factor variants and HS in CTE in dominant, additive, or recessive models (Table 4); however, *TMEM106B rs1990622* trended toward an association with HS in CTE in a recessive model for the risk allele ($p = 0.051$, Table 4).

Modeling HS in CTE

Within those participants with CTE, we utilized structural equation modeling to assess the direct and total effects of RHI, age, and CTE stage on hippocampal sclerosis and hippocampal TDP-43 inclusions. To limit the heterogeneity of sports exposure and because most participants played American football, the analysis was limited to football players. The presence of hippocampal TDP-43 inclusions had the largest direct and total effect size on hippocampal sclerosis ($\beta = 0.454$, $p < 0.01$) (Supplementary

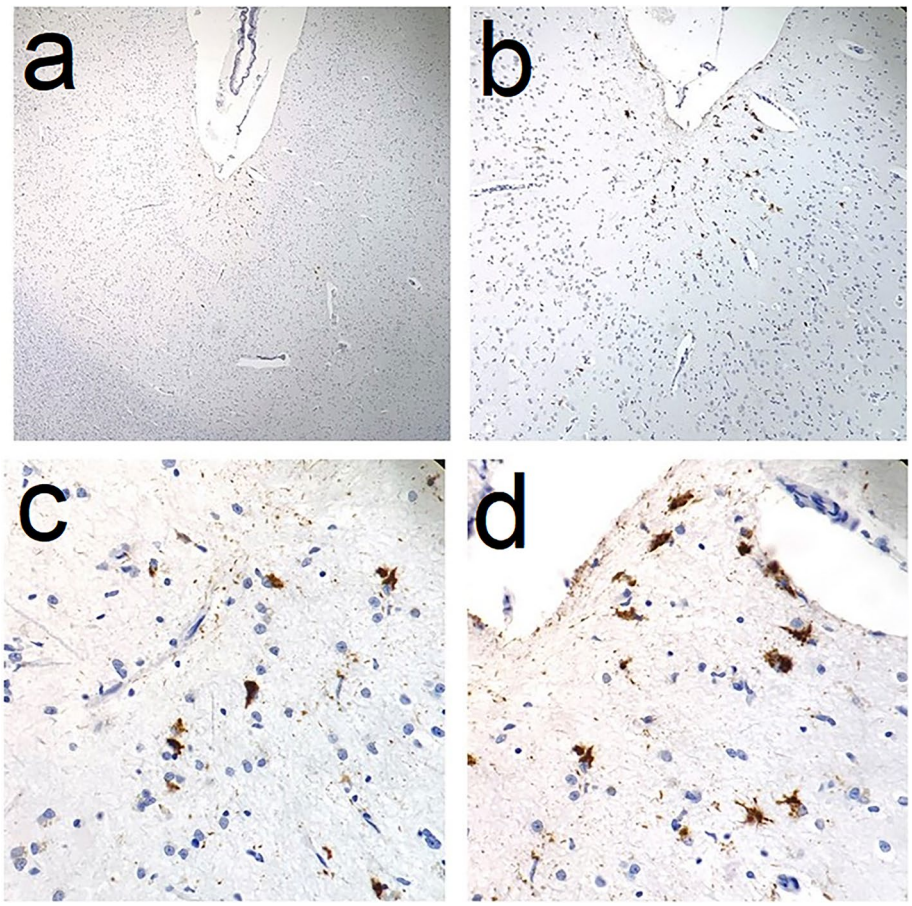

Fig. 3 Immunohistochemistry for TDP-43 in the dorsolateral frontal cortex in CTE at low power (**a**, **b**) showing involvement restricted to the depth of a sulcus. At higher power, perivascular glial and neuronal involvement can be observed (**c**, **d**)

Table 2, online resource). Additionally, both age ($\beta = 0.146$, $p = 0.01$) and CTE stage ($\beta = 0.129$, $p = 0.03$) contributed directly to hippocampal sclerosis. Years of football play did not have a significant direct effect on hippocampal sclerosis but instead was directly associated with CTE stage ($\beta = 0.237$, $p < 0.001$) and indirectly with hippocampal TDP-43 ($\beta = 0.155$, $p = 0.001$). There was a total effect of age ($\beta = 0.402$, $p < 0.01$) and CTE stage ($\beta = 0.304$, $p < 0.01$) on hippocampal TDP-43 (Supplementary Table 2, online resource). A sensitivity analysis incorporating genetic variation in *TMEM106B* showed similar results. These results are graphically expressed in Fig. 5, showing the role that RHI, age, and TDP-43 inclusions play in the development of HS.

Stratifying age in the model

We noted that most participants with CTE/HS had an age of death in their seventies (Supplementary Table 1, online resource). To further test the effects of age, we performed sensitivity analyses stratifying our groups to participants with CTE under age 75 and, separately, those 75 and older. In the group under 75 years of age, there were similar effects to the total sample, including a direct effect of CTE stage on hippocampal TDP-43 ($\beta = 0.318$, $p = 0.004$) and a direct effect of CTE stage on HS ($\beta = 0.194$, $p = 0.102$). In the group ages 75 and above, CTE stage no longer had a significant effect on hippocampal TDP-43 or the development of HS. Because people die for various reasons, we performed a sensitivity analysis examining the age of cognitive onset, which was most frequent in those with CTE/HS in their sixties (Supplementary Table 1, online resource). When we stratified our groups based on the average age of cognitive onset at 65 years, we found that the relationships and effect sizes were similar to those using age at death.

Discussion

Both hippocampal sclerosis and TDP-43 inclusions occurred frequently in former contact sport athletes with CTE (HS: 23.4%; TDP-43: 43.3%). TDP-43 inclusions were more likely to be present in the frontal cortex and at the sulcal depths and occurred both with and without limbic involvement in CTE. Participants with CTE and HS had a higher CTE stage and more years of contact sports play than those without HS. An overall model demonstrated that age, hippocampal TDP-43, and CTE stage ($p < 0.001$) were directly associated with hippocampal sclerosis. Furthermore, RHI

Table 3 Regional hippocampal TDP43 prevalence and severity in hippocampal sclerosis with and without CTE

Regions	HS/No CTE	CTE/HS	*p* value
Subiculum, *n*	33	35	
Prevalence (%)	97.0%	97.1%	0.966
Severity mean (SEM)	1.86 (0.13)	1.69 (0.12)	0.360
CA1, *n*	33	91	
Prevalence (%)	97.0%	69.2%	**0.003**
Severity mean (SEM)	2.08 (0.14)	1.61 (0.01)	**0.012**
CA2, *n*	32	39	
Prevalence (%)	78.8%	89.7%	0.783
Severity mean (SEM)	1.46 (0.13)	1.84 (0.12)	**0.044**
CA3, *n*	33	39	
Prevalence (%)	81.8%	84.6%	0.751
Severity mean (SEM)	1.34 (0.12)	1.29 (0.10)	0.835
CA4, *n*	33	39	
Prevalence (%)	84.8%	89.7%	0.531
Severity mean (SEM)	1.31 (0.13)	1.45 (0.11)	0.452
Dentate gyrus, *n*	33	94	
Prevalence (%)	90.9%	60.6%	**0.002**
Severity mean (SEM)	1.36 (0.16)	1.86 (0.16)	**0.015**

Prevalence of TDP-43 in hippocampal subregions is given as percentage of the group. Participants with hippocampal sclerosis without TDP-43 were excluded. Estimated means and standard error (SEM) of the TDP-43 severity score for those with TDP-43 inclusions in the given region were calculated using analysis of covariance (ANCOVA) adjusting for age

p Values were determined with Chi-squared analysis for frequency data and ANCOVA adjusting for age for severity scores; $p < 0.05$ in bold

CTE Chronic Traumatic Encephalopathy, *CA* cornu ammonis

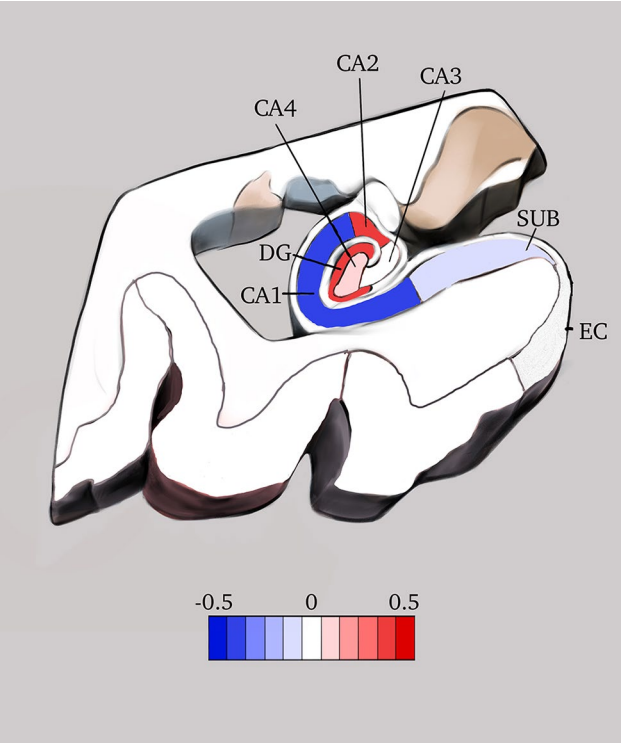

Fig. 4 Heat map of the differential severity score of TDP-43 inclusions in the hippocampal subfields of CTE-HS compared to HS alone. All cases had independent scores for each corresponding region (rated 0–3). The group estimated means are based on those with TDP-43 pathology in the given region and were calculated using analysis of covariance (ANCOVA) adjusting for age. Differences range from − 0.5 to 0.5 in the corresponding region and are based on the CTE/HS group compared to HS/No CTE group seen in Table 3. Blue indicates that the CTE/HS group has a lower average score; Red indicates CTE/HS has a higher average score than HS/No CTE in that region. *DG* dentate gyrus, *CA* cornu ammonis, *Sub* subiculum, *EC* entorhinal cortex, *TR* transentorhinal region

exposure years ($p < 0.001$) were associated with hippocampal TDP-43 through increased CTE stage.

Hippocampal sclerosis in CTE was strongly associated with the presence of hippocampal TDP-43 inclusions. A small number of cases showed HS without TDP-43, and half of these had severe tau pathology related to CTE, suggesting that rarely CTE alone may account for HS. TDP-43 pathology in CTE was predominantly limbic; however, TDP-43 inclusions were much more likely to involve the frontal lobe than in HS alone. This is consistent with previous reports of TDP-43 pathology in small numbers of CTE cases, in addition to work with *drosophila* exposed to repetitive head impacts [4]. For instance, King et al. previously reported three cases of CTE with neocortical TDP-43 inclusions [28], and HS with TDP-43 inclusions was recently reported as a common comorbidity in nine participants with a history of RHI [6].

On the other hand, within CTE, some cases did not fit the LATE-NC staging progression from the medial temporal lobe to the frontal cortex [7]. Instead, these participants had frontal cortical TDP-43 but lacked medial temporal lobe TDP-43 inclusions, suggesting that RHI or CTE tau pathology may influence the frequency and distribution of TDP-43 deposition to prematurely involve the neocortex. This frontal cortical TDP-43 pathology was often present or worse in the sulcal depths and occurred together with CTE tau pathology. Therefore, frontal cortical TDP-43 inclusions in the absence of limbic involvement may be a feature of CTE, particularly when there is a predilection for the sulcal depths.

The pattern of TDP-43 within the hippocampus appeared to differ in CTE/HS compared to HS alone. The CA1 region of the hippocampus has been shown to be selectively vulnerable to TDP-43 inclusions in HS of aging [22, 23]. Within the CTE/HS group, CA1 was less likely to be involved, while CA2 was more likely to have more severe TDP-43 pathology (Table 3, Fig. 4). CA2 is a region with early tau pathology in CTE [8] and, therefore, may be more susceptible to the effects of RHI. On the other hand, the relatively small samples sizes and high frequency of FTLD-TDP in

Table 4 Genetic variant frequencies in participants with CTE with and without hippocampal sclerosis

Characteristic	CTE/No HS	CTE/HS	Dominant *p* value	Additive *p* value	Recessive *p* value
APOE ε4 allele number					
0, *n* (%)	153 (62.6)	46 (56.8)	0.104	0.078	0.264
1, *n* (%)	78 (32.8)	30 (37.0)			
2, *n* (%)	7 (2.9)	5 (6.2)			
TMEM106B rs1990622					
CC (%)	37 (21.9)	8 (16.7)	0.972	0.164	0.051
CT (%)	89 (52.7)	18 (37.5)			
TT (%)	43 (25.4)	22 (45.8)			
ABCC9 rs1914361					
AA *n* (%)	63 (39.6)	22 (32.8)	0.715	0.630	0.656
AG *n* (%)	67 (42.1)	28 (41.8)			
GG *n* (%)	29 (18.2)	17 (25.4)			
GRN SNP rs5848					
CC (%)	26 (36.6)	5 (25.0)	0.687	0.806	0.299
CT (%)	36 (50.7)	14 (70.0)			
TT (%)	9 (12.7)	1 (5.0)			

Dominant, additive, and recessive models using the putative risk alleles were tested for associations with HS in CTE with binary logistic regression adjusting for age

CTE chronic traumatic encephalopathy, *APOE* apolipoprotein E (risk allele *ε4*), *TMEM106B* transmembrane protein 106B (risk allele T), *ABCC9* ATP-binding cassette transporter sub-family C member 9 (risk allele G), *GRN* granulin precursor (risk allele T)

CTE may also influence these findings. Future studies may help determine whether these patterns can help discriminate CTE-related HS from typical age-related HS.

Genetic risk may alter the risk for developing HS and development of LATE-NC in conjunction with CTE; however, we looked at four of the most known risk factors for development of HS and LATE-NC [17] and did not find significant differences between groups (Table 4). Variation in *TMEM106B* has previously been shown to effect the severity of CTE as well as the risk for HS. We found that the presence of the homozygous risk allele for *TMEM106B* trended toward an association with HS in CTE. However, when adding *TMEM106B* risk allele status to our model, we found similar effects for RHI on HS, suggesting that this genetic risk factor does not strongly modify the environmental risk. Variation in *APOE, ABCC9,* or *GRN* were not statistically different between CTE and CTE-HS groups, but larger studies will be necessary to confirm these findings. Further studies with greater numbers will also be required to determine whether there are gene and environment (e.g., RHI) interactions on HS.

Limbic or neocortical Lewy body disease and FTLD were relatively frequent in CTE/HS. Traumatic brain injury and RHI have both been associated with Parkinson Disease (PD) [12, 18, 44] and Lewy body disease [1]. LBD is a common comorbid pathology and can occur at younger ages than TDP-43 deposition in community aging populations [26]. In addition, there was a relatively large number of CTE participants that met criteria for FTLD-TDP (23% in CTE/HS and 6% overall). Most of the TDP-43 inclusions were intraneuronal cytoplasmic and short dystrophic neurites in the superficial layers consistent with FTLD, type A. However, investigations into the type and pattern of TDP-43 deposition in CTE with FTLD and associations with RHI require more cases and further study.

Although TBI and RHI may often co-occur, there are clear differences in later life risk between those with a history of a single TBI alone versus RHI. For instance, Johnson et al. demonstrated a lack of association between a single moderate/severe TBI and the development of TDP-43 inclusions [25]. Additionally, Crane et al. showed that pooled

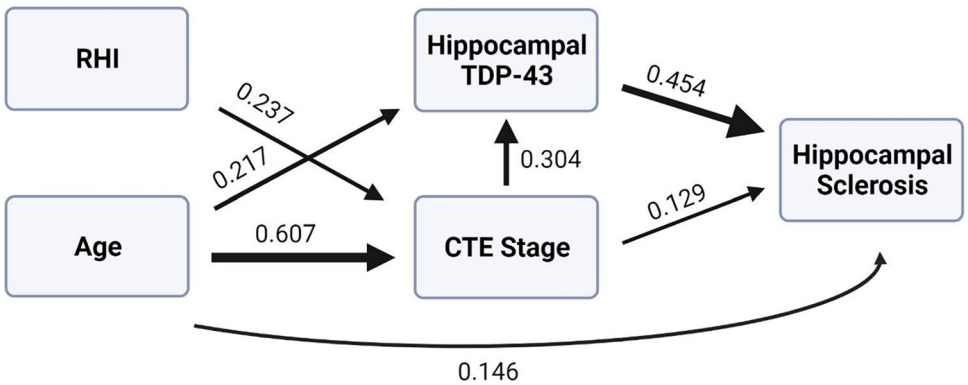

Fig. 5 Graphic representation of the structural equation modeling exploring relationships of risk factors contributing to hippocampal sclerosis. The model demonstrates that repetitive head impacts (RHI), age, CTE stage, and hippocampal p-TDP-43 inclusions all contribute to hippocampal sclerosis. All numbers shown are significant beta values. RHI uses American football years only. *RHI* repetitive head impacts, *CTE* Chronic Traumatic Encephalopathy

neuropathologic data from three cohorts indicate that a TBI with loss of consciousness is not associated with increased risk for dementia, neuritic plaques, or neurofibrillary tangles [10]. Here, we suggest that repetitive head impacts can influence the development of HS and TDP-43 inclusions in the hippocampus.

Structural equation modeling demonstrated previous and novel associations with hippocampal TDP-43 pathology and HS. In addition to the known effects of age and TDP-43 on HS, RHI and CTE stage also had significant associations with the presence of HS. We also demonstrated that years of American football participation were indirectly associated with hippocampal sclerosis via hippocampal TDP-43 and CTE stage (Fig. 5). Overall, this indicates that repetitive head impacts may be a risk factor for the development of TDP-43 inclusions and hippocampal sclerosis. As expected, age was strongly associated with HS in CTE. However, the average age at death was significantly younger in CTE/HS than in our HS alone comparison group and what has been reported in the literature for community aging populations [3, 46, 49]. When we stratified by age, the associations between CTE stage and hippocampal TDP-43 and HS were similar in both young and older groups. Thus, RHI or CTE might accelerate the development of HS as described for other neurodegenerative pathologies [1, 56, 57].

A recent study of 13 community and population-based longitudinal studies across the United States, United Kingdom, Brazil, Austria, and Finland looked at over 6000 participants and found that 39.4% of community autopsy series had LATE-NC of any stage, with an average age of death of 88.1 years [48]. We find that 43.3% of our CTE participants would have met the criteria for LATE-NC despite a much younger age of death, 62.2 years. The higher frequency and much younger age of death further suggest that RHI's may be a risk factor for TDP-43 inclusions which might accelerate LATE-NC staging. Although the type and pattern of TDP-43 in CTE is indistinguishable from LATE-NC in many cases, the increased numbers of individuals with frontal cortical involvement, including a subset with frontal only inclusions at the sulcal depths, suggest that TDP-43 deposition in CTE is at least partially independent of the typical age-related TDP-43 inclusions. It is therefore prudent to be cautious when applying LATE-NC diagnosis criteria to younger individuals [47], especially if they have a history to RHI. As noted in a recent update to the LATE-NC guidelines, TDP-43 inclusions in the context of a history of brain trauma or CTE may not represent LATE-NC and should be interpreted with caution [51]. We suggest that if TDP-43 is found with a predilection for sulcal depths in the frontal cortex that this be considered a part of CTE and not LATE-NC.

There are several limitations to the present study. Brain donors were largely recruited via self-selection or next-of-kin referral, which introduces autopsy-based selection bias that may hinder generalizability. However, inverse probability weighting recently demonstrated that study selection did not significantly affect the relationship between RHI and CTE pathology [30, 41]. The average age of the ADRCs was much older than that of the UNITE brain bank, which may account for the older age of death of the HS/No CTE group. The vast majority of the groups are Caucasian men, further limiting generalizability. Future studies within larger community-based aging cohorts with RHI history as well as prospective studies will be necessary to confirm and expand these findings.

In conclusion, we show that in addition to age and genetic variation, repetitive head impacts and advanced CTE may be risk factors for the development of hippocampal sclerosis. Therefore, HS and TDP-43 are important considerations in patients with a history of RHI and possible CTE. Future studies examining molecular mechanisms underpinning the effects of RHI on hippocampal TDP-43 inclusions and HS are warranted.

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Data availability Data used in this study is available from the Boston University Alzheimer's Disease Center (https://www.bu.edu/alzresearch/information-for-investigators/) and from the authors by request.

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