# Chronic Effects of Mild Neurotrauma: Putting the Cart Before the Horse?

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## Abstract

Accumulation of phosphorylated tau (p-tau) is accepted by many as a long-term consequence of repetitive mild neurotrauma, based largely on brain findings in boxers (dementia pugilistica) and, more recently, former professional athletes, military service members, and others exposed to repetitive head trauma. The pathogenic construct is also largely accepted and suggests that repetitive head trauma (typically concussions or subconcussive forces) acts on brain parenchyma to produce a deleterious neuroinflammatory cascade, encompassing p-tau templating, trans-synaptic neurotoxicity, progressive neurodegenerative disease, and associated clinical features. Some caution before accepting these concepts and assumptions is warranted, however. The association between history of concussion and findings of p-tau at autopsy is unclear. Concussions and subconcussive head trauma exposure are poorly defined in available cases and the clinical features reported in CTE are not at present distinguishable from other disorders. Because control groups are limited, the idea that p-tau drives the disease process via protein templating or some other mechanism is preliminary. Much additional research in CTE is needed to determine if it has unique neuropathology and clinical features, the extent to which the neuropathologic alterations cause the clinical features, and whether it can be identified accurately in a living person.

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## Introduction

The literature on chronic effects of mild neurotrauma has expanded substantially in the last decade following the description of chronic traumatic encephalopathy (CTE) in some National Football League (NFL) players. Public health concerns fostered in part by descriptive neuropathology, and in part by sensationalistic media exposure, have prompted many studies relating to the sequelae of athletic exposure, as well as broad speculation about disease mechanisms. The ostensible overlap with neurodegenerative diseases in terms of pathology and pathogenesis has fueled speculation, as well as concern.

In 1928, Martland noted that some boxers, boxing promoters, and fans were aware of a "peculiar condition" described as "punch drunk," but there had been no medical research or documentation on the deleterious effects of boxing on the brain (1). This punch drunk syndrome, also called dementia pugilistica (2) and chronic traumatic encephalopathy (3), was described in case studies for 40 years. In 1969, Roberts published a book entitled *Brain Damage in Boxers: A Study of the Prevalence of Traumatic Encephalopathy Among Ex-Professional Boxers* (4). This book provides detailed clinical information on a random sample of 224 retired professional boxers, 11% of whom were deemed to have mild CTE and 6% were considered to have a moderate-to-severe form of the syndrome. These boxers had an enormous exposure to neurotrauma, with many having hundreds of professional fights. Based on his analysis of this random case series of boxers, Roberts described the syndrome as predominately cerebellar or extrapyramidal, typically characterized by dysarthria and motor problems, with some cases having dementia.

The description of the neuropathology and clinical features of CTE has evolved over the past 7 years, and there are differences in how it has been characterized by the 2 primary research groups that have reported on the autopsy cases. The authors have described fairly extensive gross pathological and microscopic features as being characteristic of or associated with CTE. As these case descriptions have evolved, however, the collective data suggest that the only consistent hallmark of CTE is abnormal phosphorylated-tau (p-tau) accumulation (5-7). Clinical correlates are diverse and include depression, anger dyscontrol, suicidality, mild cognitive impairment, parkinsonism, dementia, and motor neuron disease; this has obscured a definable clinical phenotype at this time. Neuropathological findings also vary considerably. Although the pattern or regional distribution of p-tau in localized "epicenters" (e.g. depths of sulci, perivascular areas of cerebral cortex, and superficial cortical laminae) is cited as diagnostic of CTE, the threshold for the CTE pattern appears low: any extent of p-tau within epicenters (5), or even "sparse" p-tau according to another group (8), is considered to be indicative of CTE. Extensive medial temporal lobe and brainstem involvement is prominent in some depicted cases, although such involvement occurs in other tauopathies as a function of age, and in the recently described primary age-related tauopathy (9). Axonal varicosities in the deep cortex and subcortical white matter and co-labeling of p-tau lesions with TDP-43 are also reported (5), although these may not have the same specificity for CTE compared to the regional distribution of p-tau.

Four stages of CTE have been described by the Boston University group (5), with p-tau accumulation increasing as a function of stage. The average age of affected patients also

increases with increasing stage. The group that reported the second largest series in football players alternatively notes four "phenotypes," none of which parallel the four stages described by the Boston University group (8),.

The suspected initiator of the disease in most reported cases is exposure to American football and boxing, although CTE has also been attributed to soccer (10), baseball, rugby, hockey (5), wrestling (11), blast injury (12), protracted head banging in the setting of self-injurious autism (13), frequent falls in the setting of epilepsy (10), and dwarf tossing (14). The clinical presentation and progression of CTE are described in detail elsewhere (5, 15). In reported case series, a minority of individuals with the characteristic pathological findings of CTE may be asymptomatic during life or have nonspecific symptoms such as headaches. Others are reported to have a range of psychiatric problems including depression, impulsivity, aggression, explosive anger, mismanagement of personal circumstances, and suicidality. Cognitive impairment, including short-term memory loss, word-finding difficulty, and executive dysfunction, in addition to later-stage dementia, has also been reported in some cases. Interestingly, of all autopsy cases reported to date in NFL athletes, all but 2 were reported to have CTE, and those 2 without CTE were asymptomatic athletes in their twenties (5, 8). The autopsy cases to date are obviously not representative of all retired NFL players because the vast majority of retired players do not appear to have clinical evidence of dementia, parkinsonism, or a neuropsychiatric condition or disease. In an epidemiological study, former NFL players were reported to have a higher rate of dementia as a contributing cause of death than is expected in the general population (16); however, in terms of absolute numbers only 2.1% (7/334) of those who had died had dementia listed on their death certificates as a contributing cause of death, and only 0.9% (3/334) had Parkinson disease listed as a contributing cause of death. In a phone survey of a stratified random sample of 1,063 retired NFL players (17), they were asked if they had ever been diagnosed with "dementia, Alzheimer's disease, or other memory related disease." Of those between the ages of 30–49 years, 1.9% said yes (compared to 0.1% of men in the US general population) and for those aged over the age of 50 years, 6.1% said yes (compared to 1.2% of men in the general population).

### Pathogenesis of CTE

An emerging proposed mechanism for disease pathogenesis warrants some brief and general discussion. The mechanism begins with mild traumatic brain injury (i.e. mild TBI, concussion, or repetitive subconcussive neurotrauma) and proceeds through neurodegeneration. At the outset, it should be emphasized that the individual mechanisms are incompletely understood at each step in the process.

Our understanding of mild TBI neuropathology in the acute state is limited by the paucity of human material, although one study examining the brains of individuals who died from other causes shortly after mild TBI noted evidence of axonal injury, suggesting that mild TBI shares pathogenic mechanisms with diffuse traumatic axonal injury (18). Primate models of the physics of diffuse traumatic axonal injury have demonstrated the importance of certain biomechanical variables, such as rotational acceleration, coronal plane acceleration, and low strain rate (19, 20). This basic framework for TBI biomechanics,

however, has been superseded in recent years by data on the biochemistry of TBI, obtained through murine and cellular models (21, 22). Such models have markedly expanded the molecular pathogenesis of TBI, much of which has been referred to as "neuroinflammation." Mild TBI is thus said to result from biomechanical forces that act on parenchymal tissue to induce neuroinflammation, and includes mechanisms that span a spectrum of molecular biology (23-29).

The presence of p-tau at autopsy in the brains of former athletes has prompted investigations into a trauma-induced, neuroinflammatory basis for p-tau deposits and disease pathogenesis. Moreover, the finding of p-tau in athletes has led to subsequent experimental studies that propose a direct link between acute trauma and neurofibrillary degeneration (12, 30). The complexities inherent in relating tau phosphorylation with acute events, however, are substantial. For instance, tau protein has some 79 possible phosphorylation sites, about 20 of which have been shown to be phosphorylated in Alzheimer disease (AD) (31, 32). Kinase-phosphatase biology differs according to phosphorylation site, as do the specificity of a number of anti-phospho-tau antibodies (32). Soluble and insoluble tau isoform profiles also vary as a function of disease phenotype. Phosphorylation is temporally heterogeneous in disease and, importantly, varies physiologically as a function of developmental stage (33). In this context, the simple presence of p-tau, as detected by any single antibody, is limited as an indicator of acute tau biology in vivo. It should also be viewed alongside p-tau accumulation purely as a function of age, starting in childhood (34). The overall conclusion, however, that acute trauma leads to chronic deposition of p-tau, is embedded in the literature (12, 30). More research in this area is needed.

It is noteworthy that p-tau in disease is now hypothesized to engage in protein templating with trans-synaptic transmission, reminiscent of concepts in prion diseases. Transgenic mice overexpressing mutant tau show accumulation of wild-type p-tau in synaptically connected brain regions (35). Prion-like conformers of tau have also been shown to appear along neuroanatomical pathways following experimental inoculation (36). Comparable strain variation of amyloid-β has been demonstrated experimentally, and is suggested to comprise the basis for phenotypic variability of AD (37, 38). More research is needed, however, to determine whether the in vitro data are applicable to neurodegeneration in humans.

The presumed pathogenesis of p-tau accumulation in athletes extends further to clinical correlation, because involvement of frontal and temporal circuitry by the neurotoxic process correlates anatomically with neurobehavioral centers involved in disordered impulse control, explosive aggressiveness, extreme impulsivity, impaired judgment and social function, and heightened suicidality. Medial temporal lobe and brainstem involvement, as depicted in advanced stage cases of CTE, may in turn disrupt episodic memory and the many potential functions associated with these brain regions (39).

### Limitations of the Evolving Concepts: The Biomechanical Substrate of P-tau Is Unknown

Although p-tau accumulation of varying morphologies and distributions is reported in trauma models (12), the biomechanical substrate of p-tau lesions in the postmortem human brain has not been elucidated. It is reasonable to speculate that neurotrauma may predispose to such lesions given the variable description of changes in boxers and American football

players, but at present there is minimal evidence that concussion or subconcussive blows cause the p-tau lesions described in CTE. Interestingly, in one experimental model in which blunt trauma was delivered to the thorax (only), perivascular inflammatory changes were noted within parenchymal brain tissue via a hydrodynamic pulse mechanism (40). This raises the additional possibility that chronic neuroinflammation may occur with trauma apart from head trauma per se (e.g. increased intrathoracic pressure from impact to the chest or abdomen), and suggests again that further study is needed before drawing conclusions about specific biomechanical mechanisms or clinical substrates such as concussion/subconcussion. At present, it can be safely concluded that the distribution of p-tau in the brain at autopsy does not predict whether a given decedent suffered mild TBI or repetitive neurotrauma some years before. Nor does mild TBI or extent of subconcussive exposure predict the extent and distribution of p-tau.

## The Literature to Date

Dementia pugilistica has long been recognized and is often cited as synonymous with the modern descriptions of CTE. In recent autopsy series, dementia pugilistica is included among CTE cases (5, 8). In a recent comprehensive review of all reported cases, however, Gardner et al noted differences between dementia pugilistica, which the authors refer to as "classical" CTE, and recently reported cases, or "modern" CTE (15). Among the differences were numbers of reported cases, age at onset, age at death, sport exposure, frequency of progression, clinical features, likelihood of having an apolipoprotein E (*APOE*) ε4 allele, and neuropathological features. Noteworthy among the neuropathological features of dementia pugilistica were medial temporal neurofibrillary degeneration out of proportion to plaque pathology, substantia nigra degeneration, and cerebellar scaring; these features are variable or not present at all in modern CTE cases. Features regarded as specific for modern CTE, such as p-tau accumulation in depths of sulci, p-tau in superficial cortical laminae, and perivascular p-tau have been reported in a small number of cases of dementia pugilistica cases (10, 13).

Nevertheless, because modern CTE is presumed to be a mechanistic parallel to dementia pugilistica, some features of dementia pugilistica are worth noting. The dementia pugilistica literature is based on either case reports or retrospective series with numerous complicating factors. For example, the first appearance of "punch drunk" syndrome in the literature was based on a group of boxers reported to a forensic pathologist by a single boxing promoter, of which neuropathological findings were available in only one case (1). Brandenberg and Hallervorden were the first to call attention to neurofibrillary pathology in a 51-year-old boxer; however, this one subject had extensive plaque pathology as well as cerebral amyloid angiopathy (41), and, therefore, likely had early onset AD. In 1962, Courville reported an autopsy case of punch drunk syndrome but neuropathological findings were nonspecific; they included age-related gross atrophy, meningeal fibrosis, and "lipoid" deposits with fine and coarse granulation in large striatal neurons (42). In 1967, Constantinides and Tissot described severe degeneration of the substantia nigra with numerous neurofibrillary tangles in a 58-year-old man who had been retired from boxing for 34 years, which raises the possibility of a sporadic corticobasal syndrome (43). In 1968, Payne described autopsy findings in 6 boxers in their forties and noted cavum septum pellucidum and septal

fenestrations, now recognized as common in both dementia pugilistica and CTE (44). Interestingly, Payne reported that "a small number of senile plaques were seen in the cortex of one brain (case 1) and early neurofibrillary changes were observed in two brains (case 3 and 5). These changes indicate non-specific degenerative phenomena."

The neuropathology of dementia pugilistica was codified by Corsellis et al in 1973 in the largest series of boxers to date (n = 15); they noted as common features: (i) neurofibrillary degeneration out of proportion to plaque pathology, (ii) septal abnormalities, (iii) substantia nigra degeneration, and (iv) cerebellar scarring. Clinical findings often included speech abnormalities and movement disorders (43). Noteworthy, however, were numerous co-morbidities, including alcohol abuse, exposure to motor vehicle accidents, hypertensive cerebrovascular disease with lacunar infarcts, neurosyphilis, and a cavernous malformation in the globus pallidus. Moreover, a subset of cases had no neurological signs and no significant pathological findings. Still others were re-examined in subsequent years using amyloid-beta (Aβ) immunohistochemistry and found to have frank AD (45, 46). Cases reported since Corsellis et al are few, but have pointed out neurofibrillary tangles in the cerebral cortex in asymptomatic young boxers who died acutely, superficial layer p-tau pathology in 2 cases, and perivascular p-tau pathology in one case (10, 13). The superficial layer and perivascular p-tau in those cases are consistent with the modern descriptions of p-tau accumulation in CTE. One case of apparent dementia pugilistica with motor neuron disease was reported in a boxer, although this subject also had a family history of motor neuron disease (47). Therefore, it appears that dementia pugilistica is associated with increased p-tau in the brain in most reported cases, but the spectrum of clinical and pathological changes have not been fully defined alongside control cases matched for sport exposure.

Modern CTE is also defined retrospectively although better characterized pathologically. McKee and colleagues have raised the discussion to a new level, with an industrious brain procurement effort, accumulation of a number of brains of athletes and others exposed to neurotrauma, as well as rigorous neuropathological case characterization (5). Self-selection and associated biases is axiomatic in autopsy brain studies, so the issue of prevalence and relevant control samples will continue to be raised. Nevertheless, the findings, particularly those demonstrated by whole mount, thick immunostains, placed emphasis not solely on tau pathology per se, but on the regional distribution of the tau pathology, which had been alluded to in a small number of cases in the dementia pugilistica literature.

Both dementia pugilistica as classically defined and modern day CTE are, therefore, limited to self-referred retrospective series or case reports. Clinical manifestations, co-morbidities, and neuropathology all span a wide spectrum from absence of findings to advanced disease. Disease progression is doubtful or has not been reported in many cases. Because the definition of a neurodegenerative disease requires (i) a clinical substrate, (ii) disease progression typically leading to advanced dementia or death, and (iii) a pathological substrate at autopsy, CTE may, at present, fall short of this nosological category.

## Lack of Genetic Susceptibility Factors in CTE

Because CTE encompasses some cases with no clinical symptoms during life, other cases with nonspecific neuropathological findings, and other cases still with no evidence of disease progression, identifying genetic susceptibility will be challenging. This is particularly true for NFL athletes because nearly all athletes with NFL exposure examined at autopsy meet criteria for CTE. Without further stratification of case material on a pathogenically meaningful basis, uncovering genetic susceptibility might be mathematically precluded. Nevertheless, apolipoprotein E genotype (*APOE*) is a logical starting point, given its role as the major genetic risk for sporadic AD. In the largest CTE series to date, *APOE* allelic frequencies were comparable to the general population (5). An over-representation of the ε4 allele in dementia pugilistica was noted in one study, although the definition of dementia pugilistica in that study was purely clinical, with no autopsy correlation (48).

Some have noted similarities between the clinical presentation of CTE and that of frontotemporal dementia (FTD) (49). Moreover, the pattern and distribution of TAR DNA-binding protein-43 in cases of dementia pugilisitica resembles that of cases of FTD (50). Given the expanding list of pathogenic mutations in FTD, and also the increasingly recognized FTD–amyotrophic lateral sclerosis (ALS) spectrum, genetic analysis could be employed to refine the CTE diagnosis by excluding those cases with disease-causing mutation. At present, familial and sporadic ALS have identifiable pathogenic mutations in 37% and 6% of cases, respectively, while familial and sporadic FTD have mutations in 21% and 6%, respectively (51). More common mutations include microtubule-associated protein tau (*MAPT*), progranulin (*GRN*), and *C9ORF72*. Less common mutations include those in TAR DNA-binding protein-43 (*TARDP*), valosin-containing protein (*VCP*), p62/ sequestosome-1 (*SQSTM1*), ubiquilin 2 (*UBQLN2*), superoxide dismutase-1 (*SOD1*), and charged multivesicular body protein 2B (*CHMP2B*). Among known mutations, linkages to microtubule-associated protein tau (*MAPT*) and progranulin (*GRN*) are largely restricted to an FTD presentation, and superoxide dismutase-1 (SOD1) mutations present as ALS (52). Mutations involving C9ORF72, *TARDP, VCP, SQSTM1, UBQLN2*, and *CHMP2B* may have mixed FTD-ALS phenotypes.

Because it accounts for a substantial percentage of familial ALS and FTD cases, particularly in Europeans, *C9ORF72* may be particularly relevant to CTE (53). Mutation involves expanded hexanucleotide repeats on the non-coding portion of chromosome 9, and is now the most common mutation in both ALS and FTD. It would be interesting to explore whether the cases of apparent CTE with co-morbid ALS have expanded *C9ORF72*, and whether long-term neurological outcome in collision sports varies as a function of repeat length.

## P-tau as a Mediator of Human Disease

Whether p-tau in the human brain is toxic or protective, or neither, is unclear. Its accumulation in a broad range of conditions including normal aging indicates a secondary role in many disease states (54). Likewise, an abundance of evidence supports p-tau as a disease response, possibly even a beneficial disease response (55, 56). In an elegant study, Santacruz et al further demonstrated that neurodegeneration in transgenic mice expressing

mutant tau (P301L) could be reversed by suppressing the tetracycline promoter, and yet neurofibrillary tangles continued to accumulate in the face of improvements in memory (57).

The kinetics of p-tau in CTE during life are also unclear. Whether a non-progressive equilibrium is reached, whether p-tau is elaborated and degraded, whether p-tau forms a nidus for local progression, or whether it is some combination of the 3, is not established. The overlay of the normal aging process, which itself is associated with progressive tau pathology, is an additional consideration. P-tau involvement of anterior-medial temporal lobe and brainstem tegmentum, for example, is an expected finding in even young controls (58). The term "tauopathy," often invoked to describe CTE and other processes, may be unfortunate in light of these data in that it conveys the presumption that p-tau drives the disease, when in fact p-tau may be a downstream response.

The tau templating concept, which in essence is the non-Mendelian transmission of negative phenotypic information, is intriguing in light of parallel concepts in prion diseases. Extending these concepts to tauopathies, however, may be premature. The evidence at present is entirely experimental, requiring fundamentally aberrant, pathogenic mutations, expression of (or inoculation of) supraphysiologic concentrations of mutated or insoluble, phosphorylated protein, complex behavioral outcomes, and imperfectly modeled pathological changes. It should also be noted that p-tau, as detected by AT8 immunohistochemistry, is found in the locus coeruleus as early as childhood (34). The locus coeruleus in turn has extensive, even "unsurpassed" connections throughout the cerebrum (59). Taken together, these basic human data argue against spreading tauopathy as an in vivo disease mechanism. More research in this area is needed.

## Structure Is a Poor Predictor of Function in Neurologic Diseases

It should be pointed out that severe neuropsychiatric disturbances occur in the absence of currently identifiable neuropathological lesions, and substantial neuropathological alterations found at autopsy often go clinically unnoticed. Even in the case of AD, in which basic, measurable cognition and rigorously quantitated hallmark lesions may be compared, pathology is a modest predictor of clinical disease. Indeed, only end-stage pathology is a reliable predictor of dementia in AD (60). Moreover, blinded neuropathologic examination cannot reliably and accurately distinguish dementia in persons over 80 years from the cognitively intact (61). Other clinical distinctions, such as clinical variants of frontotemporal dementia (e.g. behavioral variant vs. primary progressive aphasia variants), and Lewy body dementia versus Parkinson disease, are often not accurately predicted on the basis of specific proteinopathy or regional neuropathology (51, 62). Drawing mechanistic associations between proteinopathy at autopsy and complex behaviors such as depression, suicide, and explosive anger is therefore problematic, and even more so given that former NFL athletes have been shown to have a decreased risk of suicide (63, 64) and explosive anger (17) compared to men in the general population.

## Directions for Future Research

The recent postmortem descriptions of the gross and microscopic neuropathology associated with CTE include changes associated with aging, neurotrauma, and a variety of neurological

and neurodegenerative diseases. For many readers who are not neuropathologists, it appeared as if all of the described features were characteristic of the disease, and, until recently, it was not clear which of these neuropathological features was considered unique to CTE. Some of the neuropathological findings attributed to CTE can occur in cognitively normal individuals, and it often coexists with pathologic changes in other diseases. Comorbidities such as AD, Lewy body disease, and cerebrovascular disease, and the nature and extent of other neuropathologic changes (e.g. TAR DNA-binding protein-43 abnormalities) should be documented carefully in future studies using detailed and specific neuropathological staging for comorbid diseases (65, 66).

The lack of clarity and specificity in the neuropathological features is also present in the clinical features. CTE has been diagnosed at postmortem examination in former athletes with no clinical features, headaches, depression, mild cognitive impairment, dementia, and motor neuron disease. The modern cases of CTE have been written as if the unique neuropathology (i.e. epicenters of p-tau) is the cause of complex changes in behavior such as depression, suicidality, anger control problems, gambling, and mild cognitive impairment. Suicide is a good example of the complexity of this issue. Virtually all of the articles relating to CTE published since 2010 have asserted that suicide is a core clinical feature of the disease. Prior to these recent cases, over the past 80 years, suicide was not reported as a core clinical feature. In fact, in the random sample of 250 retired boxers studied by Roberts (4), there were no confirmed cases of suicide (although there was one case of carbon monoxide poisoning), and in an epidemiological study of causes of death in former NFL players, the rate of suicide was lower than the rate for men in the general population (63). Two independent reviews of the literature have concluded that there is insufficient evidence to conclude that CTE is a risk factor for suicide (64, 67).

Research to date suggests that the only consistent hallmark of recent cases of CTE is abnormal p-tau accumulation in regionally specific areas (e.g. perivascular and depths of sulci) (5-7). It is not known whether this tau pathology, especially in small amounts, can cause depression, substance abuse, suicidality, personality changes, or cognitive impairment. Notably, extensive hemispheric sampling from front to back with whole mount, sledge microtome, free floating sections (50 to 100 μm) immunostained for tau (with AT8) maximizes sensitivity for detecting lesions and should be used in all cases and controls. Given that AT8 is a clean and robust monoclonal antibody, and the accumulations are dense and insoluble with strong antigenicity, it is important to study control subjects with the same methodology. That will allow researchers to determine whether any of the regionally specific tau depositions occur in people who have no history of repetitive neurotrauma. Therefore, it will be important for researchers to (i) establish clearly defined neuropathological criteria for CTE, (ii) develop coding and reporting procedures for the gross and microscopic features that are not unique to CTE, (iii) agree on and codify regions of interest, sampling, staining techniques, and procedures for reporting of results, including clinicopathologic correlations, such as is done in AD, (iv) conduct neuropathological studies of control subjects of men in their fifties and sixties who have a history of chronic depression, substance abuse, and cardiovascular disease, but no known history of neurotrauma or participation in contact sports, and (v) conduct neuropathological studies of

control subjects of men in their fifties or sixties with a history of neurotrauma, but no signs of neurologic or psychiatric problems during life.

## Conclusions

Three major questions with regard to the chronic effects of mild neurotrauma should be considered: (i) the relationship between concussions and subconcussive neurotrauma exposure and the described clinical syndrome, (ii) the relationship between concussions and subconcussive neurotrauma exposure and p-tau accumulation, and (iii) the relationship between the clinical syndrome and p-tau accumulation. The null hypothesis, or the default position, states that there is no relationship between measured phenomena. With respect to CTE, the null hypothesis is applicable and should be tested because relationships between these variables have not been demonstrated statistically, despite widely published inferences that concussions lead to both the broadly defined clinical syndrome and a unique distribution of p-tau. Moreover, variables that might be compared are incompletely defined. Concussion is not quantified in a meaningful way in the autopsy cases, the clinical syndrome described in CTE has not been shown to reliably differ from other psychiatric and neurological conditions, and the specificity of p-tau accumulation for disease has yet to be substantiated alongside control subjects with CTE-like symptoms and problems but no history of mild neurotrauma or participation in contact sports, or asymptomatic exposure-matched controls.

While fundamental questions remain unanswered, recent studies appear directed not towards verifying the existence of CTE as a neuropathologically or clinically specific disease, but in detailing the molecular cascade responsible for the clinical features, pathology, and biochemistry. This approach may be traced to the mid-1980's, when lately available molecular techniques identified protein constituents of AD lesions, and then pathogenic mutations associated with those protein constituents, prompting the idea that lesions were cause rather than consequence; in effect, driving the discussion beyond basic unanswered questions, and putting the "cart before the horse" in progress toward therapeutic intervention. The failure of small-molecule based therapeutic trials in AD to date, however, has since served as a reminder of the challenges, and that there is much to learn about the fundamental relationship between biology and pathology, not only in neurodegenerative diseases, but in the evolving entity of CTE.

## Acknowledgments

## References

1. Martland HS. Punch drunk. J Am Med Assoc. 1928; 19:1103–7.

2. Millspaugh JA. Dementia pugilistica. US Naval Medicine Bulletin. 1937; 35:297–303.

3. Critchley, M. Hommage a Clovis Vincent. Paris: Maloine; 1949. Punch-drunk syndromes: The chronic traumatic encephalopathy of boxers.

4. Roberts, A. Brain damage in boxers: A study of prevalence of traumatic encephalopathy among ex-professional boxers. London: Pitman Medical Scientific Publishing Co.; 1969.

5. McKee AC, Stein TD, Nowinski CJ, et al. The spectrum of disease in chronic traumatic encephalopathy. Brain. 2013; 136:43–64. [PubMed: 23208308]

6. McKee AC, Cantu RC, Nowinski CJ, et al. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J Neuropathol Exp Neurol. 2009; 68:709–35. [PubMed: 19535999]

7. McKee AC, Daneshvar DH, Alvarez VE, et al. The neuropathology of sport. Acta Neuropathol. 2014; 127:29–51. [PubMed: 24366527]

8. Omalu B, Bailes J, Hamilton RL, et al. Emerging histomorphologic phenotypes of chronic traumatic encephalopathy in American athletes. Neurosurgery. 2011; 69:173–83. discussion 183. [PubMed: 21358359]

9. Crary JF, Trojanowski JQ, Schneider JA, et al. Primary age-related tauopathy (PART): a common pathology associated with human aging. Acta Neuropathol. 2014; 128:755–66. [PubMed: 25348064]

10. Geddes JF, Vowles GH, Nicoll JA, et al. Neuronal cytoskeletal changes are an early consequence of repetitive head injury. Acta Neuropathol (Berl). 1999; 98:171–8. [PubMed: 10442557]

11. Omalu BI, Fitzsimmons RP, Hammers J, et al. Chronic traumatic encephalopathy in a professional American wrestler. J Forensic Nurs. 2010; 6:130–6. [PubMed: 21175533]

12. Goldstein LE, Fisher AM, Tagge CA, et al. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. Science translational medicine. 2012; 4:134ra160.

13. Hof PR, Knabe R, Bovier P, et al. Neuropathological observations in a case of autism presenting with self-injury behavior. Acta Neuropathol. 1991; 82:321–6. [PubMed: 1759563]

14. Williams DJ, Tannenberg AE. Dementia pugilistica in an alcoholic achondroplastic dwarf. Pathology. 1996; 28:102–4. [PubMed: 8714284]

15. Gardner A, Iverson GL, McCrory P. Chronic traumatic encephalopathy in sport: a systematic review. Br J Sports Med. 2014; 48:84–90. [PubMed: 23803602]

16. Lehman EJ, Hein MJ, Baron SL, et al. Neurodegenerative causes of death among retired National Football League players. Neurology. 2012; 79:1970–4. [PubMed: 22955124]

17. Weir, DR.; Jackson, JS.; Sonnega, A. National Football League Player Care Foundation: study of retired NFL players. Ann Arbor, MI: University of Michigan, Institute for Social Research; Sep 10. 2009

18. Blumbergs PC, Scott G, Manavis J, et al. Staining of amyloid precursor protein to study axonal damage in mild head injury. Lancet. 1994; 344:1055–6. [PubMed: 7523810]

19. Adams JH, Graham DI, Gennarelli TA. Head injury in man and experimental animals: neuropathology. Acta Neurochir Suppl (Wien). 1983; 32:15–30. [PubMed: 6581702]

20. Gennarelli TA. Head injury in man and experimental animals: clinical aspects. Acta Neurochir Suppl (Wien). 1983; 32:1–13. [PubMed: 6581696]

21. Morrison B 3rd, Elkin BS, Dolle JP, et al. In vitro models of traumatic brain injury. Annu Rev Biomed Eng. 2011; 13:91–126. [PubMed: 21529164]

22. Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. Nat Rev Neurosci. 2013; 14:128–42. [PubMed: 23329160]

23. Begum G, Yan HQ, Li L, et al. Docosahexaenoic acid reduces ER stress and abnormal protein accumulation and improves neuronal function following traumatic brain injury. J Neurosci. 2014; 34:3743–55. [PubMed: 24599472]

24. Cornelius C, Crupi R, Calabrese V, et al. Traumatic brain injury: oxidative stress and neuroprotection. Antioxid Redox Signal. 2013; 19:836–53. [PubMed: 23547621]

25. Balan IS, Saladino AJ, Aarabi B, et al. Cellular alterations in human traumatic brain injury: changes in mitochondrial morphology reflect regional levels of injury severity. J Neurotrauma. 2013; 30:367–81. [PubMed: 23131111]

26. Woo SK, Kwon MS, Ivanov A, et al. The sulfonylurea receptor 1 (Sur1)-transient receptor potential melastatin 4 (Trpm4) channel. J Biol Chem. 2013; 288:3655–67. [PubMed: 23255597]

27. Giunta B, Obregon D, Velisetty R, et al. The immunology of traumatic brain injury: a prime target for Alzheimer's disease prevention. J Neuroinflammation. 2012; 9:185. [PubMed: 22849382]

28. Wong J, Hoe NW, Zhiwei F, et al. Apoptosis and traumatic brain injury. Neurocritical care. 2005; 3:177–82. [PubMed: 16174891]

29. Tran HT, Sanchez L, Brody DL. Inhibition of JNK by a peptide inhibitor reduces traumatic brain injury-induced tauopathy in transgenic mice. J Neuropathol Exp Neurol. 2012; 71:116–29. [PubMed: 22249463]

30. Lucke-Wold BP, Turner RC, Logsdon AF, et al. Linking traumatic brain injury to chronic traumatic encephalopathy: identification of potential mechanisms leading to neurofibrillary tangle development. J Neurotrauma. 2014; 31:1129–38. [PubMed: 24499307]

31. Buee L, Bussiere T, Buee-Scherrer V, et al. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Brain Res Rev. 2000; 33:95–130. [PubMed: 10967355]

32. Augustinack JC, Schneider A, Mandelkow EM, et al. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. Acta Neuropathol. 2002; 103:26–35. [PubMed: 11837744]

33. Mandelkow EM, Mandelkow E. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. Cold Spring Harbor perspectives in medicine. 2012; 2:a006247. [PubMed: 22762014]

34. Braak H, Thal DR, Ghebremedhin E, et al. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. J Neuropathol Exp Neurol. 2011; 70:960–9. [PubMed: 22002422]

35. de Calignon A, Polydoro M, Suarez-Calvet M, et al. Propagation of tau pathology in a model of early Alzheimer's disease. Neuron. 2012; 73:685–97. [PubMed: 22365544]

36. Clavaguera F, Akatsu H, Fraser G, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. Proc Natl Acad Sci U S A. 2013; 110:9535–40. [PubMed: 23690619]

37. Watts JC, Condello C, Stohr J, et al. Serial propagation of distinct strains of Abeta prions from Alzheimer's disease patients. Proc Natl Acad Sci USA. 2014; 111:10323–8. [PubMed: 24982139]

38. Stohr J, Condello C, Watts JC, et al. Distinct synthetic Abeta prion strains producing different amyloid deposits in bigenic mice. Proc Natl Acad Sci U S A. 2014; 111:10329–34. [PubMed: 24982137]

39. Peskind ER, Brody D, Cernak I, et al. Military- and sports-related mild traumatic brain injury: clinical presentation, management, and long-term consequences. J Clin Psychiatry. 2013; 74:180–188. quiz 188. [PubMed: 23473351]

40. Simard JM, Pampori A, Keledjian K, et al. Exposure of the thorax to a sublethal blast wave causes a hydrodynamic pulse that leads to perivenular inflammation in the brain. J Neurotrauma. 2014; 31:1292–1304. [PubMed: 24673157]

41. Brandenburg W, Hallervorden J. Dementia pugilistica with anatomical findings. Virchows Arch. 1954; 325:680–709. [PubMed: 13226712]

42. Courville CB. Punch drunk. Its pathogenesis and pathology on the basis of a verified case. Bulletin of the Los Angeles Neurological Society. 1962; 27:160–8. [PubMed: 14023454]

43. Corsellis JA, Bruton CJ, Freeman-Browne D. The aftermath of boxing. Psychol Med. 1973; 3:270–303. [PubMed: 4729191]

44. Payne EE. Brains of boxers. Neurochirurgia (Stuttg). 1968; 11:173–88. [PubMed: 5729752]

45. Roberts GW, Allsop D, Bruton C. The occult aftermath of boxing. J Neurol Neurosurg Psychiatry. 1990; 53:373–8. [PubMed: 2191084]

46. Tokuda T, Ikeda S, Yanagisawa N, et al. Re-examination of ex-boxers' brains using immunohistochemistry with antibodies to amyloid beta-protein and tau protein. Acta Neuropathol. 1991; 82:280–5. [PubMed: 1759560]

47. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 12-1999. A 67-year-old man with three years of dementia. N Engl J Med. 1999; 340:1269–77. [PubMed: 10210712]

48. Jordan BD, Relkin NR, Ravdin LD, et al. Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. JAMA. 1997; 278:136–40. [PubMed: 9214529]

49. Andrikopoulos J, Montenigro PH, Stern RA. Clinical presentation of chronic traumatic encephalopathy. Neurology. 2014; 83:1991–3. [PubMed: 25404647]

50. King A, Sweeney F, Bodi I, et al. 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. 2010; 30:408–19. [PubMed: 20102526]

51. Rademakers R, Neumann M, Mackenzie IR. Advances in understanding the molecular basis of frontotemporal dementia. Nat Rev Neurol. 2012; 8:423–34. [PubMed: 22732773]

52. Bennion Callister J, Pickering-Brown SM. Pathogenesis/genetics of frontotemporal dementia and how it relates to ALS. Exp Neurol. 2014; 262:84–90. [PubMed: 24915640]

53. Liu Y, Yu JT, Sun FR, et al. The clinical and pathological phenotypes of frontotemporal dementia with C9ORF72 mutations. J Neurol Sci. 2013; 335:26–35. [PubMed: 24090760]

54. Goedert M, Spillantini MG. Pathogenesis of the tauopathies. J Mol Neurosci. 2011; 45:425–31. [PubMed: 21785996]

55. Castellani RJ, Nunomura A, Lee HG, et al. Phosphorylated tau: toxic, protective, or none of the above. J Alzheimers Dis. 2008; 14:377–83. [PubMed: 18688087]

56. Trojanowski JQ, Lee VM. Pathological tau: a loss of normal function or a gain in toxicity? Nat Neurosci. 2005; 8:1136–7. [PubMed: 16127446]

57. Santacruz K, Lewis J, Spires T, et al. Tau suppression in a neurodegenerative mouse model improves memory function. Science. 2005; 309:476–81. [PubMed: 16020737]

58. Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. Acta Neuropathol. 2011; 121:171–81. [PubMed: 21170538]

59. Jones BE. Noradrenergic locus coeruleus neurons: their distant connections and their relationship to neighboring (including cholinergic and GABAergic) neurons of the central gray and reticular formation. Prog Brain Res. 1991; 88:15–30. [PubMed: 1813920]

60. Nelson PT, Braak H, Markesbery WR. Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship. J Neuropathol Exp Neurol. 2009; 68:1–14. [PubMed: 19104448]

61. Brayne C, Richardson K, Matthews FE, et al. Neuropathological correlates of dementia in over-80-year-old brain donors from the population-based Cambridge city over-75s cohort (CC75C) study. J Alzheimers Dis. 2009; 18:645–58. [PubMed: 19661624]

62. Goldman JG, Williams-Gray C, Barker RA, et al. The spectrum of cognitive impairment in Lewy body diseases. Mov Disord. 2014; 29:608–21. [PubMed: 24757110]

63. Baron SL, Hein MJ, Lehman E, et al. Body mass index, playing position, race, and the cardiovascular mortality of retired professional football players. Am J Cardiol. 2012; 109:889–96. [PubMed: 22284915]

64. Iverson GL. Chronic traumatic encephalopathy and risk of suicide in former athletes. Br J Sports Med. 2014; 48:162–5. [PubMed: 24178363]

65. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. 2012; 123:1–11. [PubMed: 22101365]

66. Hyman BT, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement. 2012; 8:1–13. [PubMed: 22265587]

67. Wortzel HS, Shura RD, Brenner LA. Chronic traumatic encephalopathy and suicide: a systematic review. BioMed Res Internat. 2013; 2013:424280.