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SPINAL CORD PATHOLOGY IN
CHRONIC TRAUMATIC ENCEPHALOPATHY
WITH MOTOR NEURON DISEASE

by

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SPINAL CORD PATHOLOGY IN CHRONIC TRAUMATIC ENCEPHALOPATHY WITH MOTOR NEURON DISEASE

BRIAN FRY

ABSTRACT

Chronic Traumatic Encephalopathy (CTE) is a neurodegenerative disease caused by repetitive head trauma and mild traumatic brain injuries (mTBIs) and has been associated with contact sports such as football, boxing, and ice hockey. CTE is a slowly progressing neurological disease that is often clinically associated with symptoms of memory loss, decline in cognitive function, behavioral changes such as increased impulsivity and aggression, and/or suicidal thoughts. Advanced stages of the disease present with more severe neurological changes such as dementia, speech and gait abnormalities, and parkinsonism.

Amyotrophic Lateral Sclerosis (ALS, also known as Lou Gehrig Disease) is a progressive and fatal neurodegenerative disease characterized by motor neuron loss and corticospinal tract degeneration. While 90-95% of ALS cases are sporadic in nature, many genetic mutations have been identified that contribute to familial forms of the disease. The etiology of sporadic ALS is unknown but it is likely caused by a complex interaction of various genetic and environmental risk factors. Epidemiological evidence suggests that one such risk factor is brain trauma, the main risk factor associated with the development of CTE.

In this study the spinal cord tissue of twelve athletes diagnosed with CTE who also developed a progressive motor neuron disease and showed symptoms of profound
muscle weakness, atrophy, spasticity, and fasciculations several years before death was examined. The spinal cord tissue from these 12 CTE cases with motor neuron disease (CTE+MND) was compared to the spinal cord tissue of 10 sporadic ALS control cases. Results showed a difference in frequency of tau pathology between the two disease cohorts, as one-third of CTE+MND cases and none of the ALS cases showed tau immunoreactivity. In addition, TDP-43 immunoreactivity was present in every CTE+MND case but one and was present in all ALS cases. Motor neuron inclusions were positive for both FUS and p62 in both cohorts, and no distinct differences were observed cystatin C pathology.

Overall, this suggests that the spinal cord inclusions in CTE+MND have a similar composition to sporadic ALS. However, there is an increased frequency of tau pathology in CTE+MND though this result did not reach statistical significance in this study.
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LIST OF ABBREVIATIONS

ALS ...................................................................................... Amyotrophic Lateral Sclerosis
CPCS .................................................................................. Chronic Post Concussion Syndrome
CTE ...................................................................................... Chronic Traumatic Encephalopathy
CTE+MND ............................................................................ Chronic Traumatic Encephalopathy with Motor Neuron Disease
DTI ........................................................................................ Diffuse Tensor Imaging
fALS ...................................................................................... Familial Amyotrophic Lateral Sclerosis
fMRI ........................................................................................ Functional Magnetic Resonance Imaging
FUS ....................................................................................... Fused in Sarcoma Protein
MND ....................................................................................... Motor Neuron Disease
NFT ....................................................................................... Neurofibrillary Tangle
p62 ........................................................................................ Nucleoporin p62 protein
p-tau ...................................................................................... Hyperphosphorylated Tau Protein
sALS ...................................................................................... Sporadic Amyotrophic Lateral Sclerosis
SIS ....................................................................................... Second Impact Syndrome
SOD1 ...................................................................................... Copper-Zine Superoxide Dismutase 1
TBI ....................................................................................... Traumatic Brain Injury
TDP-43 .................................................................................. TAR DNA-Binding Protein 43
INTRODUCTION

Concussions and Traumatic Brain Injury (TBI)

While no uniform definition for concussion currently exists, the consensus statement from the 2012 International Conference on Concussion in Sport held in Zurich defines a concussion as a pathophysiological process resulting in functional neurological impairments as a consequence of forceful biomechanical impacts directly on or transmitted to the head, neck, or face (McCrory et al., 2013). Brain injury follows from rapid acceleration and deceleration mechanics that occur during a traumatic impact and can include linear, translational, and/or rotational forces, with translational and rotational forces thought to be the most significant contributors toward the severity of injury (Kimpara and Iwamoto, 2012; McCrory et al., 2013; Zhang et al., 2004). Symptoms vary from person to person, but they typically include some signs of physical injury (such as headache, dizziness, or nausea), signs of cognitive impairment (such as memory loss, difficulty concentrating, or confusion), and/or behavioral changes (such as emotional lability, fatigue, or anxiety) (Jordan, 2013). While conditions such as second impact syndrome (SIS) have thrown the media spotlight on the immediate dangers of concussions and traumatic brain injuries, illnesses such as Chronic Traumatic Encephalopathy (CTE) and chronic post concussion syndrome (CPCS) have elucidated the possible long-term dangers of head trauma.
Epidemiology of Concussion and Brain Injury

While figures vary, it's estimated that 1.6-3.8 million sports-related TBIs occur annually in the United States alone (Langlois et al., 2006). However, this number is likely an underestimate of the overall incidence of concussions as it does not include data from outpatient facilities, the U.S. Military, nor those who choose not to seek medical care after injury (Coronado et al., 2012). Though the incidence of concussion seems to have been increasing in recent years, it may be in part because of better concussion awareness, education, and diagnosis (Daneshvar et al., 2011). Significant risk factors for sustaining a sports-related concussion include having a history of a previous concussion, playing in a match vs. practice, and type of sport played (i.e. collision vs. non-collision). Lower level risk factors include being younger in age, being female, and level of play (i.e. high school, college, or professional), among others (Abrahams et al., 2013).

The media has brought the health concerns over sports-related brain injury into the public eye with its recent coverage of the ex-football players’ 2013 settlement with the National Football League regarding the dangers of on-field head injuries, as well as the airing of the 2013 PBS Frontline documentary “League of Denial,” which provides an in-depth analysis of head trauma in the NFL (and is based off of the book by the same title). Although much exciting research is being done in the field of traumatic brain injury, we still do not have a definitive diagnostic tool to assess concussions nor do we currently have a sophisticated treatment protocol for people who suffer these injuries. Diffuse tensor imaging (DTI) and functional magnetic resonance imaging (fMRI) are promising tools that can help detect subtle injuries within the brain and may aid in
concussion diagnosis; however the standard treatment protocol typically only involves immediate removal from physical and cognitive stimuli until symptoms resolve (Jordan, 2013). While some physicians will prescribe medications to treat severe symptoms associated with brain injury (headache, dizziness, anxiety), treatment does not currently exist to repair and/or reverse any damage done to the brain at the molecular level.

**Second Impact Syndrome (SIS)**

SIS is a rare but potentially fatal condition that occurs when an athlete receives a second traumatic head injury while still symptomatic from a previous concussion. The second traumatic hit leads to diffuse brain swelling, cerebral edema, and eventually brain herniation, which is thought to arise from the loss of autoregulation of cerebral blood flow (Jordan, 2013). The exact frequency of SIS is unknown but one U.S. study reported 17 deaths from SIS over a 30-year period (Thomas, 2011). Given the mortality and the severity of the injuries associated with the syndrome, SIS is a major concern for athletes who suffer from an acute concussion and necessitates a conservative approach to recovery before returning to a contact sport such as American football or ice hockey.

**Chronic Post Concussion Syndrome (CPCS)**

CPCS is an uncommon condition in which an athlete experiences long-term or permanent post-concussion symptoms following a traumatic brain injury. While no consensus criteria exist for CPCS, the term generally refers to acute post-concussion symptoms that last longer than one year as a result of a single concussive event (Jordan,
2013). Common symptoms include headache, dizziness, impaired attention, memory problems, executive dysfunction, irritability, and depression and often result in the athlete retiring from sport. While the pathological mechanisms underlying CPCS are unknown, it represents a condition that results directly from a single concussive event, thus clearly distinguishing it from neuropathological diseases such as Chronic Traumatic Encephalopathy and Alzheimer’s disease, where repetitive head trauma over a period of years or even decades may trigger neurodegeneration.

**Head Trauma and Neurodegenerative Disease**

There is mounting evidence for a causal link between head trauma and the development of neurodegenerative diseases such as Chronic Traumatic Encephalopathy, Alzheimer’s disease, Parkinson’s disease, ALS, and frontotemporal dementia (Gardner and Yaffe, 2014; Walker and Tesco, 2013). Studies suggest that levels of amyloid-beta protein (the pathological protein most often associated with Alzheimer’s disease) are raised following acute brain trauma and it’s been shown that pathological hyperphosphorylated tau accumulation (the pathological protein most often associated with CTE) is significantly higher in individuals with a history of repetitive head trauma (Mielke et al., 2014; McKee et al., 2013). Due to the nature of this thesis, epidemiological, clinical, and pathological information will only be given for CTE and ALS.
Chronic Traumatic Encephalopathy (CTE) Overview

CTE is a slowly progressing neurodegenerative disease found in individuals with a history of repetitive brain trauma. The origins of CTE can be traced back to 1928 when a New Jersey pathologist, Harrison Martland, described a condition called “punch drunk,” a set of neurodegenerative symptoms found in boxers resulting from repeated blows to the head (Martland, 1928). Although the condition was originally coined “dementia pugilistica” in 1937 by Millspaugh, the realization that sports other than boxing were associated with its development led to the use of “progressive traumatic encephalopathy” in 1949 followed by CTE in 1957 (Millspaugh, 1937; Critchley, 1949, 1957).

Because head trauma is inherent in the nature of most contact sports, CTE has been found mostly in the brains of former amateur and professional boxers, wrestlers, American football players, ice hockey players, and rugby players; however, the disease has also been found in the brains of military explosives specialists, one former professional baseball player, and most recently in the brain of a former collegiate level soccer player (McKee et al., 2013; Mohney, 2014). It’s important to note that all reported neuropathologically confirmed cases of CTE have had exposure to repetitive brain trauma during life; however, not all subjects with a history of repetitive brain trauma develop CTE. This indicates that additional genetic and environmental risk factors likely have a role in the neuropathogenesis of the disease (Stern et al., 2013).
**Epidemiology of CTE**

Unfortunately, the body of published literature on CTE is still quite small and thus epidemiological data regarding its prevalence in the population is not available at the time of this writing. While data is available on the number of confirmed CTE cases from the Sports Legacy Institute-Boston University CTE Brain Bank, the total sample size and inherent selection bias prevent the data from being generalized to a larger population. Larger scale prospective studies are being done at the Boston’s University’s CTE Center that will eventually shed some light on CTE’s prevalence, particularly in professional American football players.

**Clinical Presentation of CTE**

Generally speaking, early symptoms associated with CTE include aggression, irritability, depression, impulsivity, short-term memory loss, and heightened suicidality that typically begin eight to ten years after experiencing repetitive traumatic brain injury. Later stages of the disease show symptoms that reflect more serious neuropathological changes and include dementia, speech and gait abnormalities, and parkinsonism (McKee et al., 2009). No methods currently exist to diagnose CTE in living subjects—the disease can only be confirmed via post-mortem neuropathological evaluation of a subject’s brain tissue. Because the clinical symptoms of CTE overlap with several other neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and frontotemporal dementia, the discovery of a promising biomarker for the disease is vital.
in order to help distinguish CTE’s clinical presentation and the ability to diagnose the disease during life.

Recently, Stern and colleagues published the largest analysis of clinical symptoms associated with CTE and suggested that there are two distinct clinical presentations of CTE: one variant that initially displays behavioral or mood changes and another that initially displays cognitive impairment (Stern et al., 2013). The behavior/mood group presented with symptoms at a significantly younger age and was also more emotionally explosive, out of control, physically and verbally violent, and depressed than the cognition group. Stern and colleagues also highlighted the trend that subjects in the behavior/mood group almost always developed cognitive impairments at some point in time but that significantly fewer subjects in the cognition group demonstrated behavior and mood changes during the course of the disease. While all subjects in the cognition group were reported to have impaired episodic memory, approximately 25% of the behavior/mood group did not have reported memory difficulties.

While it’s uncertain how these differences reflect neuropathological changes, Stern and colleagues suggest that it’s possible these differing symptoms stem from pathological changes focused in different parts of the brain during the course of the disease. Early development of tau pathology in the locus coeruleus and amygdala could play a role in the behavioral and mood changes reported for the younger group, while degeneration in the hippocampus and frontal cortex could play a role in the memory and executive dysfunction in the cognition group (Stern et al., 2013; McKee et al., 2013). Stern and colleagues also pointed out that the many of the symptoms found in the
younger behavioral/mood group could be due to persistent postconcussion syndrome with unresolved or progressive axonal damage resulting from the subjects’ initial head trauma. While selection bias and small sample size in the 2013 Stern study limit the generalizability of its conclusions to the greater CTE population, its findings certainly merit further investigation into clarifying the clinical presentation of CTE.

**Neuropathology of CTE**

The hallmark pathological feature of CTE is the widespread deposition of hyperphosphorylated tau (p-tau) in the form of neurofibrillary tangles (NFTs). Normal tau proteins are intracellular and function to stabilize neuronal microtubules, particularly within axons. Upon becoming phosphorylated, tau will dissociate from the microtubules and form pathological protein aggregates known as NFTs (and eventually lead to cell death). Recent evidence suggests that pathobiology of forming NFTs may be more harmful than the resulting NFTs themselves, as cellular abnormalities associated with their formation include a disruption in autophagy, vesicular trafficking mechanisms, axoplasmic transport, neuronal polarity, and the secretion of tau into the extracellular space (Gendreau and Hall, 2013).

While the clinical presentation of CTE may be confused with other neurodegenerative diseases, the neuropathological changes of CTE are clearly distinct from other tauopathies such as Alzheimer’s disease and frontotemporal lobar degeneration (McKee et al., 2013). According to McKee and colleagues, gross neuropathological features of CTE include atrophy of many brain structures involved in
normal cognitive and behavioral functioning, ventricular dilation, and septal abnormalities such as a splayed or even absent septum pellucidum. Additionally, extensive p-tau pathology is found in frontal and temporal cortices, preferentially clustered around small vessels at the depths of cerebral sulci, as well as in major limbic and central brain region structures such as the thalamus and hippocampus. There is also severe degeneration of axons and white matter tracts throughout the brain and brainstem. Most CTE cases also show evidence of TAR DNA-binding protein 43 (TDP-43) pathology with a relative absence of beta amyloid (the pathological protein most associated with Alzheimer’s disease and aging) (McKee et al., 2013).

In the same paper, McKee and colleagues separated the spectrum of CTE into four progressive stages. A summary of the gross and microscopic features of each stage can be found in Table 1 at the end of this section. The mildest stage of CTE (stage I) was grossly characterized by occasional mild ventricular enlargement with other gross neuropathological features and brain weights not significantly different than those of normal brains. Microscopically, stage I CTE was characterized predominantly by discrete areas of peri-vascular p-tau NFTs and astrocytic tangles, predominantly located in the depths of cerebral sulci. Scattered and distorted axonal varicosities were found in the frontal cortex as well as subcortical white matter and deep white matter tracts of the diencephalon. Additionally, roughly half of the stage I cases examined showed TDP-43 immunopositive neurites in the frontal subcortical white matter and fornix.

Gross pathology of stage II CTE cases remained fairly similar to stage I cases and included mild ventricular enlargement; however, roughly one third of cases showed a
small cavum septum pellucidum (a splaying of the membrane that separates the lateral ventricles), roughly one-third of cases showed pallor of the locus coeruleus and substantia nigra, and one case showed severe atrophy of one mammillary body.

Microscopically, multiple epicenters of p-tau NFTs and astrocytic tangles were found in the depths of cerebral sulci. In addition, p-tau NFTs were found in superficial layers of the cortex with and moderate deposition of p-tau NFTs in the nucleus basalis of Meynert and locus coeruleus. Very mild deposition of p-tau NFTs was found in medial temporal lobe structures including the hippocampus and entorhinal cortex as well as in midbrain structures including the substantia nigra and the dorsal and median raphe nuclei.

Distorted axonal varicosities were found in frontal and temporal cortices as well as white matter tracts. About 80% of the stage II cases examined showed TDP-43 immunopositivity, a minority of which showed severe TDP-43 pathology in widespread regions of the brain and spinal cord.

Grossly, stage III CTE cases showed mild cerebral atrophy with moderate ventricular dilation. A little over 40% of brains showed septal deformities including cavum septum pellucidum, septal tears, or a total absence of the septum entirely. A larger percentage of cases showed moderate depigmentation of the locus coeruleus and mild depigmentation of the substantia nigra (~60% and ~40%, respectively). Other common gross pathological features included shrinkage of the mammillary bodies and thalamus, a distinct convex shape of the medial thalamus, and thinning of the hypothalamic floor and corpus callosum. Microscopically, extensive p-tau pathology was found in medial temporal lobe structures (entorhinal cortex, hippocampus, and amygdala) and was
prominently spread in regions of the frontal, temporal, parietal, insular, and septal cortices, diencephalon, brainstem, and spinal cord. Severe axonal loss and distortions were found in subcortical white matter in the frontal and temporal cortices. A similar proportion of cases displayed TDP-43 pathology as compared to stage II cases.

Cases categorized with the most severe CTE stage (stage IV) showed increased cerebral atrophy as well as significant shrinkage of the medial temporal lobe, thalamus, hypothalamus, and mammillary bodies. In addition to having significantly smaller mean brain weights than earlier stage CTE, the majority of stage IV brains showed increased ventricular dilation, a sharply concave shape of the third ventricle, and more exaggerated septal abnormalities (cavum, perforations, or complete absence). All stage IV cases showed depigmentation of the locus coeruleus and substantia nigra where it could be assessed. Microscopically, stage IV cases showed severe neuronal loss and gliosis in the cerebral cortex, hippocampal sclerosis, and severe p-tau pathology widely distributed throughout the cerebrum, diencephalon, basal ganglia, brainstem, and spinal cord. Stage IV cases also showed severe axonal loss and distortions as well as the most severe TDP-43 pathology. TDP-43 positive neurites and inclusions were found in the cerebral cortex, medial temporal lobe, diencephalon, basal ganglia, brainstem, and less regularly in the spinal cord (McKee et al., 2013).

Motor Neuron Disease Overview

Although many disorders fall under the category of motor neuron diseases, when the term is used in a singular fashion (i.e. ‘motor neuron disease’) it generally refers to
Amyotrophic lateral sclerosis (ALS). ALS, sometimes referred to as Lou Gehrig’s disease after the famous New York Yankees baseball player, is the most common of motor neuron diseases among adults. It is a progressive and fatal disease that involves the degeneration of both upper and lower motor neurons in the brain and spinal cord.

Epidemiology of ALS

It’s estimated that the worldwide prevalence of ALS is approximately 5-7 people per 100,000 while the incidence is approximately 2 per 100,000 people (Chiò et al., 2013). About 5 to 10% of ALS cases are familial (fALS) while the remainder are sporadic (sALS). The incidence of sALS is higher in men than in women (by a ratio of about 1.5:1) and the mean onset of sALS is around 60 years of age, though about a quarter of patients present under the age of 50 years (Greenfield et al., 2008). The median survival varies but is typically around 3 years after the onset of symptoms. Longer survival has been associated with limb onset, younger age at onset, better motor function, higher breathing capacity, stable weight, and longer intervals of time between symptom onset and diagnosis of the disease (Gordon, 2013). While some research suggests that there may be an association between head trauma and ALS, other studies dispute this suspicion and thus stronger evidence is needed before claiming that head trauma is indeed a true risk factor for the development of the disease (Walker and Tesco, 2013; Armon and Nelson, 2012).
Genetics and ALS

Approximately 60% of inherited ALS cases are associated with known genetic mutations, 20% of which are caused by mutations in the copper-zinc superoxide dismutase 1 (SOD1) gene (Gordon, 2013). The SOD1 gene encodes an enzyme that destroys free superoxide radicals in the body, but when mutated the resultant protein forms toxic gain-of-function aggregates that cause neuronal loss. There’s very strong evidence to suggest pathogenic roles for other gene mutations such as transactive response DNA binding protein 43-kDa (TDP-43), fused in sarcoma protein (FUS), and C9ORF72 protein (chromosome 9 open reading frame 72), among others (Gordon, 2013). In addition, genes encoding proteins involved in the proteosomic or autophagic clearance systems, such as UBQLN2 and SQSTM1, have been associated with fALS (Verma and Tandan, 2013).

Clinical Presentation of ALS

ALS leads to the progressive degeneration of the upper motor neurons in the cerebral cortex as well the lower motor neurons in the medulla and anterior horn of the spinal cord. The general effect is progressive muscle weakness and atrophy that leads to death, usually from respiratory failure as the diaphragm and intercostal muscles lose the ability to function properly (Gordon, 2013). Lower motor neuron loss leads to muscle fasciculations, cramps, atrophy, and as a result, marked muscle weakness. Loss of upper motor neurons is associated with spasticity, hyperreflexia, and modest muscle weakness. Upper motor neuron loss can clinically manifest through the Babinski sign where a
patient has an extension reflex upon the sole of his/her foot being stroked with a blunt instrument. Emotional lability is another sign of upper motor neuron dysfunction, where a patient undergoes involuntary and uncontrollable episodes of crying, laughter, or other emotions.

ALS presents in a clinically heterogeneous manner even among family members with the disease; variations in speed of progression of the disease as well as in the origin of clinical symptoms can make it very difficult to clinically diagnose at its early stages. In addition, cognitive impairment and behavioral disturbances can also manifest in patients with the disease.

ALS begins peripherally in a patient’s limbs (usually in the arms) in about two-thirds of cases. Symptoms typically include foot drop, difficulty walking, loss of hand dexterity, or weakness when lifting the arms, and usually manifest focally and unilaterally. Bulbar-onset ALS, which is more prevalent in elderly women, carries a worse prognosis and follows the progression of symptoms from dysarthria (difficulty speaking), dysphagia (difficulty swallowing), sialorrhea (hypersalivation), to eventual malnutrition and anarthria (inability to speak) (Gordon, 2013).

Cognitive impairment was originally thought to be uncommon in ALS until fairly recently. Evidence suggests that roughly 50% of patients with ALS will present clinically with cognitive impairment and that 15% of ALS patients have severe cognitive impairment consistent with frontotemporal dementia (Phukan et al., 2007).
Neuropathology of ALS

While loss of upper and lower motor neurons is the hallmark pathological sign of ALS, no widely used system currently exists to stage the disease in a similar manner to that of CTE. Dr. John Trojanowski and colleagues published a preliminary staging system based on TDP-43 pathology and its theorized progressive spread throughout disease (Brettschneider et al., 2013). If widely adapted it would prove to be the first staging system that could be used in conjunction with neuropathological diagnosis of ALS and would constitute a major step towards understanding disease progression mechanisms. Because the staging is only preliminary, this thesis will only summarize the guidelines in brief detail.

According to Trojanowski and colleagues, stage 1 ALS cases were characterized by the presence of TDP-43 immunoreactive inclusions in the agranular motor cortex, motor neurons in the anterior horn of the spinal cord, and in bulbar motor neurons of cranial nerves V, VII, and X-XII. Stage 2 involved the pathology from stage 1 plus some involvement of the prefrontal cortex, reticular formation (both parvocellular and magnocellular portions), and in precerebellar nuclei (inferior olivary complex). Stage 3 presented with more severe TDP-43 pathology in the prefrontal cortex as well as involvement of the basal ganglia, with a particular focus within the striatum. Finally, TDP-43 pathology in stage 4 spread to the transentorhinal and entorhinal cortices as well as the hippocampal formation (Brettschneider et al., 2013).
Chronic Traumatic Encephalopathy + Motor Neuron Disease (CTE+MND)

As mentioned previously, repetitive head trauma is associated with both the development of CTE and ALS (though to much lesser degree for ALS). McKee and colleagues published a potentially ground breaking study in 2010 that provides evidence for a linkage between CTE and ALS (McKee et al., 2010). Dr. McKee and her collaborators reviewed the cases of 12 athletes with CTE, 3 of whom also had signs and symptoms of Motor Neuron Disease during their lifetimes. The study compared the brain and spinal cord pathology of these 3 cases (referred to as CTE+MND for Chronic Traumatic Encephalopathy with Motor Neuron Disease) with the pathology of the CTE-only cases, as well as age- and sex- matched ALS-only cases and normal control brains.

In the brains of cases with CTE but without MND (n=9), pathological changes of CTE were present (p-tau pathology in the frontal, temporal, and insular cortices, diencephalon, basal ganglia, and brainstem). 7 of the 9 cases displayed extensive TDP-43 immunoreactivity, with TDP-43 pathology found in the frontal and temporal cortices, insula, subcortical white matter, brainstem, amygdala, hippocampus, caudate, putamen, thalamus, and hypothalamus. In the 5 cases with spinal cord tissue available for analysis, all 5 showed tau-immunoreactive neurites and NFTs in the lateral, dorsal, and anterior horns of the spinal cord, while only 2 of 5 cases showed TDP-43 pathology in the form of occasional TDP-43 positive ring neurites.

Macroscopically, the brain and spinal cord tissue of athletes with CTE+MND (n=3) did not differ from the CTE-only cases with exception of atrophy of the ventral roots of the spinal cord. All 3 cases showed characteristic p-tau pathology consistent with
CTE but had TDP-43 pathology in a far greater density than that found in the CTE-only brain and spinal cord tissue. In the brains of CTE+MND athletes, TDP-43 pathology was concentrated in the motor cortex. General features of the CTE+MND cases included marked myelin and axonal loss with astrocytosis in the medullary pyramids and corticospinal tracts throughout the spinal cord along with moderate to profound anterior horn cell loss with atrophy and gliosis of the ventral roots. Tau-positive pathology was found in the posterior, lateral, and ventral horns, frequently surrounding degenerating anterior horn cells.

Of the 12 sporadic ALS cases examined, rare tau-positive neurites were found in only one case while TDP-43 pathology was present and abundant in every case. In the 12 control cases examined, rare tau-positive neurites were found in the ventral horn of 4 cases while no TDP-43 pathology was observed in any of the 12 cases.

The findings of the 2010 McKee study provided promising early evidence that a distinctive and widespread TDP-43 pathology is associated with CTE (7 of 9 CTE-only cases showed extensive TDP-43 pathology) and that in some individuals the pathology may become so severe that it involves the spinal cord and manifests clinically as Motor Neuron Disease. McKee and colleagues noted that the lack of tau pathology in the ALS control cases, combined with its absence in descriptive literature surrounding Motor Neuron Disease provides fairly convincing evidence that the CTE+MND cases described are not a rare tau-variant form of MND. In addition, the widespread TDP-43 pathology reported in most CTE cases provides evidence against the coincidental comorbidity of
both CTE and ALS and rather points towards some sort of continuum of TDP-43 pathology within all CTE cases.

**Specific Aims and Objectives**

The findings of the 2010 McKee paper provided the motivation to look further into the descriptive pathology of known CTE+MND cases. Of specific interest was the pathology within the anterior horn of the spinal cord, an area that is highly affected by neurodegeneration in Motor Neuron Disease. A comparative analysis of the pathology in this region among CTE+MND, CTE, ALS, and control cases would provide further information to elucidate whether the observed CTE+MND have a distinct and separate pathology from both CTE and ALS, or whether they are unique instances of comorbid CTE and ALS pathology.

Currently in 2014, there are an additional 9 CTE+MND cases to examine along with the 3 cases cited in 2010. This thesis is a preliminary report of qualitative findings associated with a larger effort to describe and quantify (when possible) the pathology of CTE+MND and its distinguishing features in comparison to CTE, ALS, and control cases.
Table 1: **Summary of CTE staging.** Information adapted from McKee et al 2013.

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<th>Gross Pathology</th>
<th>Microscopic Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage I</strong> Occasional mild ventricular enlargement, otherwise unremarkable.</td>
<td>Discrete perivascular foci of p-tau pathology concentrated at depths of cerebral sulci. Some axonal damage and very mild TDP-43 pathology when present.</td>
</tr>
<tr>
<td><strong>Stage II</strong> Mild ventricular enlargement, occasional septal defects and occasional mild depigmentation of the substantia nigra and locus coeruleus.</td>
<td>Multiple foci of p-tau pathology at depths of cerebral sulci, p-tau NFTs spread to superficial layers of cerebral cortex, nucleus basalis of Meynert, and locus coeruleus. More moderate axonal damage and moderate TDP-43 pathology present in most cases.</td>
</tr>
<tr>
<td><strong>Stage III</strong> Mild cerebral atrophy with moderate ventricular enlargement, some thinning of the corpus callosum, and atrophy of the mammillary bodies. More frequent septal defects and depigmentation of the substantia nigra and locus coeruleus.</td>
<td>More widespread p-tau pathology extending to medial temporal lobe structures, diencephalon, brainstem, and spinal cord. More severe axonal damage and similar TDP-43 pathology to stage II.</td>
</tr>
<tr>
<td><strong>Stage IV</strong> Increased cerebral atrophy and significantly lower mean brain weight than stage I and II. Marked atrophy of medial temporal lobe, mammillary bodies, hypothalamus, and thalamus. More severe ventricular enlargement (with sharply concave contour of third ventricle), more frequent and severe septal defects, and severe depigmentation of the substantia nigra and locus coeruleus.</td>
<td>Severe widespread p-tau pathology throughout the cerebrum, diencephalon, basal ganglia, brainstem, and spinal cord. Most severe axonal damage present and more widespread and severe TDP-43 pathology extending into the cerebral cortex, medial temporal lobe, diencephalon, basal ganglia, and brainstem.</td>
</tr>
</tbody>
</table>
METHODS

Subjects

Spinal cord slides from the 12 pathologically verified CTE+MND cases were cut and stained from spinal cord tissue obtained via the brain donation program at the CTE Center at Boston University’s Alzheimer’s Disease Center and the Sports Legacy Institute-Boston University Brain Bank. Brain and spinal cord tissue for each case was obtained through written consent forms for brain and spinal cord donation signed by the donor’s legal next of kin. Institutional review board approval for brain donation was obtained through the Boston University Alzheimer's Disease Center, Boston University’s Center for the Study of Traumatic Encephalopathy (now called the CTE Center at Boston University), and the Edith Nourse Rogers Bedford Veterans Affairs Medical Center. Post-mortem clinical information for each case was obtained through analysis of the donor’s medical records (per consent of the legal next of kin) as well as interviews with the donor’s close family members. Institutional review board approval for medical record review, family interviews, and neuropathological evaluation was obtained through the Boston University School of Medicine. Dr. Ann McKee of Boston University’s School of Medicine performed the neuropathological evaluation while Dr. Robert Stern of Boston University’s School of Medicine performed the clinical interviews and evaluation for each case.

Spinal cord slides for the 10 ALS control cases were cut and stained from paraffin embedded tissue blocks received from the University of Wisconsin School of Medicine.
Choice of Immunohistochemical Stains

In this initial analysis, 5 different antibodies were chosen to stain across all of spinal cord slides for the pathological versions of the following proteins: tau (AT8 antibody), TDP-43, FUS, nucleoporin p62 (p62), and cystatin C.

Inclusion of tau and TDP-43 in this analysis was critical as the pathological versions of these proteins are commonly associated with CTE and ALS, respectively. FUS and p62 are proteins that have both been found to localize to pathological inclusions within motor neurons in ALS (Lagier-Tourenne, 2010; Safa, 2011). Cystatin C is another protein of interest as its pathological aggregates have also been found to localize to degenerating motor neurons in ALS (Okamoto et al., 2008).

Immunohistochemical Preparation

Spinal cord tissue was processed and embedded in paraffin wax with a Sakura Tissue-Tek Tissue Embedding Console System. 10 μm sections were then cut from the paraffin wax embedded tissue blocks with a Leica RM2135 microtome and individually mounted on glass slides. Immunohistochemistry staining was performed following the standard lab protocol in Dr. Ann McKee’s laboratory based out of the Edith Nourse Rogers Bedford V.A. Medical Center, which is described in detail below.

Cut paraffin sections were de-paraffinized in xylene for 10 minutes, repeated 3 times, then transferred into absolute ethanol for 2 minutes, repeated twice. Sections were then immersed in methanol and hydrogen peroxide for 30 minutes, transferred into 95% ethanol for 2 minutes, and then washed under running tap water for 5 minutes. Tissue
was then pre-treated for antigen retrieval per manufacturer guidelines (if necessary, see Table 2 below for pretreatment information) followed by a wash in running tap water for an additional 5 minutes. Sections were then transferred into phosphate buffered saline (PBS, pH 7.4) then blocked with ScyTek superblock buffer (ScyTek, Logan, UT #AAA500) for 5 minutes. The sections were then covered with the desired antibody (see Table 2 for complete list) and incubated at 4°C overnight. After allowing the sections to cool back to room temperature, they were rinsed with PBS (repeated 3 times for a total of 10 minutes) and then incubated for 30 minutes at room temperature with ScyTek Antipolyvalent Biotinylated Secondary Antibody (#ABN500), followed by an additional PBS rinse (3 times, 10 minutes total). Sections were then treated with ScyTek Horseradish Peroxidase (#ABL500) and incubated for 30 minutes at room temperature, followed by a rinse with PBS (3 times 10 minutes total). Sections were then treated with 3-amino-9-ethylcarbazole (AEC) substrate (containing 20 µL ScyTek chromogen #ACD030 in 1ml buffer #ACE500) until well developed and rinsed in distilled water followed by running tap water for 5 minutes. Sections were then counterstained in Gill Hematoxylin (Vector Laboratories, Burlingame, CA, H-3401), washed in running water for 5 minutes, immersed in PBS for 2 minutes, then washed again in running water for an additional 5 minutes. Sections were then covered with a ScyTek aqueous mounting medium (#AML500), dried at room temperature overnight (or until completely dry). Finally, sections were immersed in xylene and then cover-slipped with consul-mount (Thermo Scientific, Tewksbury, MA, #9990440). See Table 2 below for information on each of the antibodies used.
Table 2: Antibody Information for Immunohistochemical Staining

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Dilution</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT8 (tau)</td>
<td>Thermo Scientific</td>
<td>1:2,000</td>
<td>No pretreatment</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>Millipore</td>
<td>1:2,000</td>
<td>HIER citrate buffer pH 6.0</td>
</tr>
<tr>
<td>FUS</td>
<td>Proteintech Group</td>
<td>1.75µL/mL</td>
<td>HIER citrate buffer pH 6.0, 10 mins</td>
</tr>
<tr>
<td>p62</td>
<td>BD Biosciences</td>
<td>1:1,0000</td>
<td>HIER tris-buffered saline (TBS) pH 9.0, 15 mins</td>
</tr>
<tr>
<td>TDP-43</td>
<td>Cosmobio USA</td>
<td>1:2,000</td>
<td>88% formic acid, 2 mins</td>
</tr>
</tbody>
</table>

Analysis

A qualitative analysis and semi-quantitative analysis of the pathology in the anterior horns of the spinal cord of both CTE+MND cases and pure ALS cases was performed. Each available spinal cord section from each slide was examined in the anterior horn at 200x magnification. For each stain, the presence of a single immunoreactive intracellular inclusion, extracellular inclusion, or neuritic thread within any anterior horn of a specific section was given a grade of mild (+). The presence of two to nine of such immunoreactive signs was given a grade of moderate (++), and ten or greater immunoreactive signs was given a grade of severe (+++). Cases without any immunoreactivity for a particular stain were marked as negative (-) (see Table 3 for summary). For cases with multiple sections of spinal cord available for analysis on a
single slide, the most advanced grade of immunoreactivity was taken as representative for the entire case. Photo examples of each severity grade were taken using SPOT Basic Image Capture software. Victor Alvarez M.D. of the Boston University School of Medicine and the Edith Nourse Rogers Bedford V.A. Medical Center helped to confirm the grade of pathology severity for each case.

**Table 3: Pathology Grading Scale for Anterior Horn of Spinal Cord**

<table>
<thead>
<tr>
<th>Pathological Grade</th>
<th>Number of immunoreactive signs (intracellular/extracellular inclusions, neuritic threads) present in anterior horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (-)</td>
<td>0</td>
</tr>
<tr>
<td>Mild (+)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (++)</td>
<td>2-9</td>
</tr>
<tr>
<td>Severe (+++)</td>
<td>10+</td>
</tr>
</tbody>
</table>
RESULTS

A total of 12 CTE+MND cases and 10 sporadic ALS cases were analyzed (see Tables 4 and 5). The average age at death was 48.4 years for the CTE+MND cohort and 56.9 years for the ALS cohort. All CTE+MND subject and eight of ten ALS subjects were male. While no data was available for athletic history in the ALS cohort, nine of the CTE+MND subjects had played high school, college or professional football, one subject had played college soccer, and two subjects had boxed professionally. Mild, moderate, and severe immunoreactivity were observed for every stain with the exception of cystatin C, where only moderate and severe immunoreactivity were observed (see Figs. 1, 2, and 3).

Four of 12 CTE+MND cases displayed tau immunoreactivity. Of those cases one was given a mild grade, two were given a moderate grade, and one was given a severe grade. No tau immunoreactivity was observed in any of the ALS cases. TDP-43 immunoreactivity was observed in 11 of 12 CTE+MND cases and in all 10 ALS cases with a roughly similar distribution of severity grades within each disease cohort. FUS immunoreactivity was observed in all 12 CTE+MND cases and in 8 of 10 ALS cases, though 2 CTE+MND cases were graded with severe pathology while grading was limited to mild or moderate in the ALS cases. All CTE+MND and ALS cases displayed p62 immunoreactivity with a roughly similar distribution of severity. 11 of 12 CTE+MND cases and 9 of 10 ALS cases displayed positive cystatin C immunoreactivity, again with a roughly similar distribution of pathology severity.
between the two diseases. No single CTE+MND or ALS case lacked immunoreactivity across all of the evaluated stains.
Figure 1: **Images of AT8 (stains for tau) and TDP-43 Pathology Staging.** AT8 (left column) and TDP-43 (right column)
Figure 2: **Images of FUS and p62 pathology staging.** FUS (left column) and p62 (right column).
Figure 3: **Images of Cystatin C pathology staging.** Mild stage (+) Cystatin C pathology was not found in any of the cases analyzed.
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Athletic hx</th>
<th>CTE stage</th>
<th>AT8</th>
<th>TDP-43</th>
<th>FUS</th>
<th>p62</th>
<th>Cyst C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>M</td>
<td>66</td>
<td>NFL football</td>
<td>3</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Case 2</td>
<td>M</td>
<td>49</td>
<td>NFL football</td>
<td>2</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Case 3</td>
<td>M</td>
<td>31</td>
<td>College football</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Case 4</td>
<td>M</td>
<td>41</td>
<td>College football</td>
<td>2</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Case 5</td>
<td>M</td>
<td>41</td>
<td>College football, boxing</td>
<td>3</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Case 6</td>
<td>M</td>
<td>67</td>
<td>NFL football</td>
<td>3</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Case 7</td>
<td>M</td>
<td>62</td>
<td>Professional boxer</td>
<td>4</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Case 8</td>
<td>M</td>
<td>32</td>
<td>HS football, police officer</td>
<td>2</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Case 9</td>
<td>M</td>
<td>62</td>
<td>Professional boxer</td>
<td>4</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Case 10</td>
<td>M</td>
<td>52</td>
<td>College football</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Case 11</td>
<td>M</td>
<td>29</td>
<td>College soccer</td>
<td>2</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Case 12</td>
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<td>2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ALS Case 1</td>
<td>M</td>
<td>43</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
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<td></td>
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<tr>
<td>ALS Case 2</td>
<td>M</td>
<td>66</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS Case 3</td>
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<td>60</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td></td>
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</tr>
<tr>
<td>ALS Case 4</td>
<td>M</td>
<td>39</td>
<td>-</td>
<td>++</td>
<td>++</td>
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<td>ALS Case 5</td>
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<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
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<tr>
<td>ALS Case 6</td>
<td>M</td>
<td>61</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>ALS Case 7</td>
<td>M</td>
<td>66</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS Case 8</td>
<td>M</td>
<td>62</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td></td>
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</tr>
<tr>
<td>ALS Case 9</td>
<td>M</td>
<td>64</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS Case 10</td>
<td>F</td>
<td>53</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td></td>
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</tr>
</tbody>
</table>
DISCUSSION

Tau Pathology

Tau is a microtubule binding protein that promotes stability within neurons. The most prominent difference between the CTE+MND and ALS slides was the presence of tau pathology in the spinal cord of one-third of the subjects with CTE+MND. This result combined with the absence of tau pathology in the ALS cases makes sense intuitively as tau pathology is a hallmark feature of CTE and is absent or extremely rare in ALS (McKee et al., 2010). While the data set is limited, the results suggest a moderate association between CTE stage and severity of tau immunoreactivity within the CTE+MND cases. The two lower stage CTE cases (both stage 2) had mild and moderate tau pathology grades, while the two higher stage CTE cases, one stage 3 and one stage 4, had severe and moderate tau pathology, respectively. The two lower stage tau-positive CTE+MND cases were also younger in age at death (31 and 41 years of age compared to 62 and 66 years of age), which may be explained by the progressive nature of tau pathology in CTE. However, none of these associations yet reach statistical significance, and more subjects are required to determine if these trends hold up.

More clinical information such as the number of documented concussions, estimated number of sub-concussive hits, and duration of playing career would be necessary to tease out any additional explanations for the presence of tau pathology in only a fraction of CTE+MND cases. Genetic analysis could also prove useful in explaining this discrepancy. Additionally, the prevalence of tau pathology in an age- and CTE stage-matched cohort of CTE-only cases would prove valuable in determining
whether the presence and distribution of spinal cord tau pathology in CTE+MND cases is unique.

TDP-43 Pathology

TDP-43 is a protein that binds both DNA and RNA and is involved in transcriptional repression, RNA splicing, and translational regulation. Hyperphosphorylated TDP-43 is pathologic in nature and has long been a marker for neurodegenerative diseases such as ALS and frontotemporal lobar degeneration (Dewey et al. 2012). As mentioned previously, a widespread TDP-43 pathology is also associated with CTE (McKee et al., 2010, 2013).

While one CTE+MND case out of twelve lacked TDP-43 pathology, the distribution of TDP-43 pathology severity grades among CTE+MND and ALS cases was fairly similar. One-third (4 of 12) of CTE+MND cases and 30% (3 of 10) of ALS cases displayed mild TDP-43 immunoreactivity. Roughly 40% (5 of 12) of CTE+MND cases and 40% (4 of 10) of ALS cases displayed moderate TDP-43 immunoreactivity, while roughly 20% (2 of 12) of CTE+MND cases and 30% (3 of 10) of ALS cases displayed severe TDP-43 immunoreactivity. Overall, abnormal TDP-43 accumulation in the spinal cord mirrors that in sporadic ALS.

FUS Pathology

Perhaps the second most prominent difference between the CTE+MND and ALS cases was observed in each cohort’s distribution of FUS pathology. FUS is an abundant
nuclear protein and performs multiple functions in RNA metabolism, but the role of FUS in the nervous system has yet to be fully characterized (Shelkovnikova, 2013). All of the CTE+MND cases displayed FUS pathology while FUS immunoreactivity was observed in only 8 of 10 ALS cases. While 2 of 12 CTE+MND cases had severe FUS pathology, no ALS cases showed more than moderate FUS pathology. However, roughly half of CTE+MND and ALS cases showed moderate FUS pathology, so it’s uncertain if the absence of severe FUS pathology in the ALS cases is the result of sampling variation. While FUS pathology has been associated with ALS (Gordon, 2013), it has not been described in CTE. Interestingly, one CTE+MND subject with FUS-positive inclusions did not have TDP-43 positivity, suggesting a possible mutation in the FUS gene in this individual.

Because of the cellular role of FUS, it’s entirely possible that FUS pathology is secondary to a larger neurodegenerative process. More research needs to be done to help illuminate the mechanisms behind pathological FUS protein aggregations and their role in neurodegenerative disease.

**p62 Pathology**

Nucleoporin p62 is a multi-domain protein that interacts with the cell’s autophagy machinery by helping tag molecules for degradation and it’s abnormal aggregates are associated with variants of both ALS and frontotemporal lobar degeneration (Bitto et al., 2014; Safa, 2011).
Very few differences in the distribution and severity of p62 pathology existed between CTE+MND and ALS cases. Both diseases showed pathological involvement of p62 across all analyzed cases, which could indicate a general disruption in the autophagy and protein clearance mechanisms.

**Cystatin C Pathology**

Cystatin C is a protein abundant in neural tissue and plays a role in many biological functions including modulation of inflammatory responses, cell proliferation and growth, and astrocytic differentiation during mouse brain development (Kaur and Levy, 2012). Cystatin C is histopathologically linked to ALS as it is one of two known proteins that localizes to neuronal inclusions called Bunina bodies within degenerating motor neurons (Okamoto et al., 2008). Levels of plasma and cerebrospinal fluid cystatin C may also prove to be a promising biomarker for ALS (Wilson et al., 2010).

Similar to p62, few differences existed in the distribution and severity of cystatin C pathology between CTE+MND and ALS cases. A slightly greater number of ALS cases (5 of 10) cases than CTE+MND cases (4 of 12) showed severe Cystatin C pathology, though this small difference is likely due to random variation. The prevalence of Cystatin C pathology in both cohorts suggests a role of general inflammation and cell damage in both disease processes.
Limitations

Brain and spinal cord tissue are particularly limited resources and can be very difficult to obtain. This fact, combined with the small number of existing neuropathologically verified CTE+MND restricts the usefulness of the associations made from the data presented in this study. This pilot study suggests that a cohort of 20-30 CTE+MND cases along with 20-30 ALS cases will be required to appropriately power the analysis for tau staining. It would also be useful to have a better age-matched ALS group as 6 of 12 CTE+MND cases were under the age of 49 (with 3 of the 6 below the age of 32) while only 2 of 10 ALS cases were under the age of 53 (and none below the age of 39).

Additionally it will be useful to have a CTE-only comparison group to separate out what differences between the CTE+MND and ALS groups could be attributed to CTE alone. This would be especially useful for determining whether the tau pathology in the CTE+MND was unique in any way and it would help establish a rough baseline prevalence for spinal cord tau pathology within the CTE population for comparison.

Another factor that likely affected results is the severity of motor neuron cell loss within a specific case. Pathology that localizes within a neuron (such as a hyperphosphorylated tau neurofibrillary tangle or a TDP-43 inclusion) will be much less observed or even absent in the anterior horn of cases with severe motor neuron degeneration. For this reason a lack of pathology or a mild severity grade according to the metrics used in this study may not be truly representative of the severity of disease.
This analysis was also skewed by the number of spinal cord sections from particular level contained on a single slide, which varied from a single section to up to six sections for any given case. Pathology grades were not always consistent across all sections on a single slide for a particular case and this sampling error was introduced by not using a set number of sections from which to make observations. Additionally, it was impossible to ensure that all spinal cord tissue was taken from roughly the same level from each case. It would be very useful to for future research to analyze representative sections at all four levels of the spinal cord (cervical, thoracic, lumbar, and sacral). This would not only reduce sampling error but may also help elucidate the mechanisms and spread of certain pathological aggregates.

**Conclusion and Future Directions**

These results demonstrate that the spinal cord pathology in CTE+MND largely parallels that in sporadic ALS. Both diseases have lower motor neuron inclusions that are positive for TDP-43 and FUS. However, there is a trend towards a greater frequency of tau pathology in CTE+MND. An additional fact that may be of importance is the discrepancy in that the average age in the CTE+MND cohort (48.4) was substantially less than the average age of the ALS cohort (56.9), which could suggest that CTE may accelerate the motor neuron degeneration when present.

The preliminary results discussed in this thesis necessitate a more advanced analysis of the spinal cord pathology in CTE+MND with additional CTE control cases and a better age-matched ALS cohort. A larger sample size and analysis of all levels of
spinal cord with a consistent number of sections from each level will refine and expand these results. It would also be useful to examine pathology within the motor cortex to examine if any relationships exist between brain and spinal cord pathology within each disease. Additionally, it would be very useful to gather in depth clinical data for all cases analyzed, including but not limited to information on symptom severity and onset, athletic history, history of head trauma, and genetic background.
REFERENCES


CURRICULUM VITAE

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• YOB: 1987

EDUCATION

Boston University
• Candidate for Masters in Medical Sciences degree, May 2014. Current GPA: 3.81

Bowdoin College
• Bachelor of Arts degree, May 2010. Economics Major, Chemistry Minor, fulfilled pre-medicine curriculum.
• Honors: Lydia Bell Award for Initiative in Public Service, Frederick G.P Thorne Award for Outstanding Male Leadership, Sarah and James Bowdoin Scholar (Dean’s List) 2008-2009, named to the 2008-2009 New England Small College Athletic Conference All-Academic Team.

WORK EXPERIENCE

Boston University’s Center for the Study of Traumatic Encephalopathy
• Research Study Assistant. Aid in the research and development of projects in Dr. Ann McKee’s laboratory surrounding cases of Chronic Traumatic Encephalopathy (CTE), a neurodegenerative disease caused by repetitive traumatic brain injury. Will write a research thesis on CTE-Motor Neuron Disease (CTE-M) to fulfill requirements for Masters degree from Boston University. Assist in coordinating tissue requests and donations, managing the brain bank, and processing incoming tissue for histological preparation. July 2013-present.

MedStar Union Memorial Hospital
• Sports Medicine Research Intern. Facilitated and coordinated efforts to create and budget an Anterior Cruciate Ligament injury prevention program for US Lacrosse. Duties included a thorough literature review and evaluation, collection and assembling of educational materials for the US Lacrosse website, and coordination across several departments. Summer 2012.

Deerfield Academy
• Health and Physics Teacher. Taught two sections of Health Issues and one section of inquiry-based ninth grade Physics. Co-created and co-taught a senior health elective that focused on the health issues involved in one’s transition to college and adult life. Served as a mentor and guide to nine student advisees. September 2010-July 2012.
• Dorm Resident and Coach. Oversaw fourteen lower class boys as a part of living in a dormitory on campus, and served as the assistant coach to boy’s varsity hockey team, the boy’s JV tennis team, and the boy’s JV football team.
• Additional Work: Created the Culture Club, a student and faculty group devoted to assessing cultural issues on campus and implementing student-led strategies to address such issues. Helped facilitate changes to the opening days schedule as well as revise the student orientation leader structure and responsibilities.
Carilion Roanoke Memorial Hospital  Roanoke, VA

- Surgery Department Research Intern. Contributed to the research of three medical studies investigating the ability of ultrasound microbubbles to blast bacteria, the relationship between rib fractures and pulmonary contusions, and the efficiency of trauma triage. Compiled and analyzed medical data, learned how to navigate medical records and read CT scans, and performed bacterial experiments in a biology laboratory. Summer 2009.
- Poster presented at the Biomedical Engineering Society in October 2010: Fry, Brian et al. The Correlation Between Rib Fractures and Pulmonary Contusions. Poster presented at: Biomedical Engineering Society Annual Meeting; 2010 Oct 6-9; Austin, TX.

The Optimal Athlete  Colorado Springs, CO

- Researched and co-wrote the following article during Summer 2008: Barnes, Mike and Brian Fry. Paradigm Shift for Ice Hockey Strength and Conditioning. The Optimal Athlete: November 2008.

VOLUNTEER WORK AND COLLEGE LEADERSHIP

bWell Center Volunteer at Boston Medical Center (BMC)  Boston, MA

- Supervised, organized, and helped run the bWell Center at Boston Medical Center, a support center for pediatric patients and their families. Provided educational assistance for patients and their families, ran daily activities promoting healthy choices for children (such as yoga and nutrition games), directed patients to appointments, and provided general support and information. January 2013-June 2013.

Co-President of BMASV (Bowdoin Men Against Sexual Violence)  Brunswick, ME

- Raised campus awareness of issues pertaining to sexual assault, sexual violence, gender, and homophobia. Organized and ran facilitations for all male athletic teams and male members of the college social houses. Learned to coordinate with other campus groups and developed public speaking and facilitation skills while dealing with important and sensitive issues. 2009-2010.

Sexual Assault Support Services of Mid-Coast Maine (SASSMM) Peer Educator  Brunswick, ME

- Educated local Maine high school, middle school, and elementary school students on issues such as sexual violence and harassment, consent, and Internet safety through facilitated discussions. Developed the necessary skills to connect with students of all ages from various backgrounds while speaking about important issues. 2009-2010.

Member of Bowdoin Men’s Varsity Ice Hockey Team & Leadership Council  Brunswick, ME

- Advise and inform the coaches on important team decisions (such as issues with lineups, team conflicts, disciplinary action) as one of five elected members of the men’s ice hockey team leadership council.
- Four-year letter winner on a nationally ranked Division III hockey program. 2006-2010

Mentor  Brunswick, ME

- Mt. Ararat Middle School and Bears and Cubs. Befriended and supported a local 6th grader and a local 4th grader through the Bridge to Kids Mentoring Program and the Big Brother Big Sister Program. 2008-2010.