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TDP-43 pathology in chronic traumatic encephalopathy

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Thesis

TDP-43 PATHOLOGY IN CHRONIC TRAUMATIC ENCEPHALOPATHY

by

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TDP-43 PATHOLOGY IN CHRONIC TRAUMATIC ENCEPHALOPATHY

DOUGLAS BARNES

ABSTRACT

Transactive response DNA-binding protein of 43 kDa (TDP-43) is the major protein found within pathological inclusions in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD) (Neumann et al., 2006). TDP-43 is a ubiquitously expressed protein mainly involved in RNA metabolism. It is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family and in its normal state is predominantly found in the nucleus. In its pathological state TDP-43 is cleaved, phosphorylated, ubiquitinated, and located in cytoplasmic or nuclear inclusions.

Along with ALS and FTLD, TDP-43 is also observed in many other neurodegenerative diseases. Pathological TDP-43 inclusions have been previously reported in cases of Chronic Traumatic Encephalopathy (CTE) (King et al., 2010)(McKee et al., 2010)(Saing et al., 2012)(Hazrati et al., 2013), however no previous study has reported on the incidence and extent of TDP-43 cellular inclusions in a large cohort of autopsy cases diagnosed with CTE.

This study finds that TDP-43 inclusions are a frequent feature of CTE, as TDP-43 inclusions are identified in 43% (20/47) of subjects in a CTE+, FTLD-, low-likelihood-of-AD cohort. Furthermore, this study finds that in CTE there is no consistent initial focus of TDP-43 pathology which spreads to neighboring regions as the disease progresses. Despite the lack of a clear progression of TDP-43 pathology, a TDP Staging
Scheme for CTE which accurately reflects the extent and severity of TDP-43 pathology in not only the study cohort, but likely in all subjects without FTLD, was established.

Four stages were identified: TDP Stage 0 showed no TDP-43 inclusions in the substantia nigra, dorsolateral frontal cortex, or dentate gyrus; TDP Stage 1 showed inclusions in either the substantia nigra or the dorsolateral frontal cortex; TDP Stage 2 showed inclusions either in the dentate gyrus or in both the substantia nigra and the dorsolateral frontal cortex; and TDP Stage 3 showed inclusions in the substantia nigra, dorsolateral frontal cortex, and dentate gyrus.

Finally, a correlation was found between the presence of TDP-43 inclusions and the levels of activated microglia in the dorsolateral frontal cortex of CTE+ subjects. This finding aligns with the theory that the pathological changes of TDP-43 found in CTE are driven by the pro-inflammatory cytokines released by chronically activated microglia.
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LIST OF ABBREVIATIONS

AD ........................................................... Alzheimer’s Disease
ALS ........................................................... Amyotrophic Lateral Sclerosis
CTE ........................................................... Chronic Traumatic Encephalopathy
CTF ........................................................... C-Terminal Fragment
CTF-25 ....................................................... C-Terminal Fragment of 25 kDa
CTF-35 ....................................................... C-Terminal Fragment of 35 kDa
ERK ........................................................... Extracellular Signal-Regulated Kinases
FL-TDP-43 .................................................. Full Length TDP-43
FTLD .......................................................... Frontotemporal Lobar Degeneration
hnRNP ....................................................... Heterogeneous Nuclear Ribonucleoprotein
JNK ........................................................... c-Jun N-Terminal Kinase
mTBI .......................................................... Mild Traumatic Brain Injury
rTBI .......................................................... Repetitive Traumatic Brain Injury
SG ........................................................... Stress Granule
TDP-43 ....................................................... Transactive Response DNA-Binding Protein of 43 kDa
INTRODUCTION

In 2006, Neumann et al. described transactive response DNA-binding protein of 43 kDa (TDP-43) as the major protein within ubiquitinated cellular inclusions found in both frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) (Neumann et al., 2006). TDP-43 cellular inclusions have since been found in a growing number of neurodegenerative diseases, including FTLD with Pick Bodies (Arai et al., 2006), Alzheimer’s Disease (Amandor-Ortiz et al., 2007), Dementia With Lewy Bodies (Nakashima-Yasuda et al., 2007), Argyrophilic Grain Disease (Fujishiro et al., 2008), Huntington Disease (Schwab et al., 2008), Progressive Supranuclear Palsy (Yokota et al., 2010), Corticobasal Degeneration (Yokota et al., 2010), and Hippocampal Sclerosis (Pao et al., 2011).

TDP-43 immunoreactivity was first reported in cases of Chronic Traumatic Encephalopathy (CTE) in 2010 (King et al., 2010). Since this original report, the finding has been confirmed by several studies, each examining a small number of cases (McKee et al., 2010)(Saing et al., 2012)(Hazrati et al., 2013). To date, no previous study has reported on the incidence and extent of TDP-43 cellular inclusions in a large cohort of autopsy cases previously diagnosed with CTE.
Normal TDP-43 Structure and Function

Figure 1: TDP-43 Structure. Adapted from Chang et al., 2015.

TDP-43 is a 414 amino acid protein encoded by the \textit{TARDBP} gene on chromosome 1 which contains the following elements (Figure 1):

- **Nuclear Localization Sequence (NLS):** Spans amino acids (AAs) 82-98 (Winton et al., 2008). Together with the Nuclear Export Sequence allows the protein to shuttle between the nucleus and cytoplasm (Mackness et al., 2014).

- **RNA Recognition Motif 1 (RRM1):** Spans AAs 106-196, necessary for normal TDP-43 function. Recognizes RNA with a minimum of five UG repeats and shows increased binding affinity to longer sequences of repeats (Tollervey et al., 2011).

- **RNA Recognition Motif 2 (RRM2):** Spans AAs 191-262, Binds shorter UG repeats with a higher affinity than RRM1 (Lukavsky et al., 2013).
- **Nuclear Export Signal (NES):** Putative NES is within RRM2 and spans AAs 236-247. Together with NLS allows the protein to shuttle between the nucleus and cytoplasm (Mackness et al., 2014).

- **Glycine Rich Domain:** Spans AAs 274-314 according to some (Ou et al., 1995), according to others spans almost the entire C-terminal after RRM2, AAs 274-414 (Pesiridis et al., 2009).

- **Prion-like Glutamine/Asparagine Rich (Q/N Rich) Domain:** Spans AAs 320-367 (Fuenteabla et al., 2010). This domain is necessary for TDP-43 autoregulation (Y. M. Ayala et al., 2011).

Primarily a nuclear protein, TDP-43 is also found in low levels in the cytoplasm (Lee et al., 2011). TDP-43 expression is not unique to the brain, as TDP-43 has been found in a variety of tissues. A 2001 study found TDP-43 to be expressed in all sixteen human tissues tested, with the highest levels found in the pancreas, placenta, spleen, ovaries, and testis (Buratti et al., 2001).

TDP-43 was originally discovered by Ou et al. in 1995 as a protein that binds TAR DNA motifs of the human immunodeficiency virus type 1 (HIV-1) (Ou et al., 1995). Further research identified it as a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family, a group of proteins mainly involved in RNA metabolism (Buratti et al., 2010). TDP-43 has numerous functions within the cell, including regulation of pre-mRNA splicing (Buratti et al., 2001), miRNA processing (Buratti et al., 2010)(Kawahara et al., 2012), mRNA stability (Volkening et al.,
2009)(Fiesel et al., 2010), mRNA transport and local translation (Wang et al., 2008), and autoregulation (Y. M. Ayala et al., 2011).

**Pathological TDP-43**

The pathological form of TDP-43 is ubiquitinated, phosphorylated, cleaved, and located in intracellular cytoplasmic inclusions (Neumann et al., 2006). When TDP-43 was originally identified as the major disease protein in FTLD-U and ALS by Neumann and colleagues in 2006 it was hoped that TDP-43 inclusions were a specific marker for those two diseases. A study published later that year began to cast doubt on this thinking, however, by reporting the finding of Pick bodies, normally characterized by the presence of pathological tau protein inclusions, with TDP-43 inclusions (Arai et al., 2006). Soon after, the findings of that study were confirmed (Freeman et al., 2008), and TDP-43 inclusions were found in many other diseases. Notably, TDP-43 inclusions are not limited to the cells of the central nervous system, as they have also been found in muscle cells of patients suffering from several types of myopathies with rimmed vacuoles (Kusters et al., 2008).

**Loss or Gain of Function?**

Three possible hypotheses have been offered up to explain how TDP-43 may cause cellular toxicity: a loss of function hypothesis, a gain of function hypothesis, and a dominant-negative hypothesis (Yang et al., 2010)(Lee et al., 2011)(Li et al., 2013)(Vanden Broeck et al., 2014). Under the loss of function hypothesis, when TDP-43
is cleared from the nucleus and localized into cytoplasmic inclusions the cell loses vital TDP-43 function and degenerates. Under the gain of function hypothesis, soluble and/or aggregated TDP-43 accumulates in the cytoplasm and gains novel toxic properties. Under the dominant-negative mechanism, TDP-43 fragments interfere with the protein’s normal function and the cell loses vital TDP-43 function. It should be noted that these processes are not mutually exclusive and a combination of two or all three may be at work (Vanden Broeck et al., 2014).

**TDP-43 Modifications Associated with Neurodegeneration**

TDP-43 within cellular inclusions may be cleaved, phosphorylated, and ubiquitinated (Neumann et al., 2006). At this time, however, the significance of these changes has not been established.

**Figure 2:** Normal vs. Pathological State of TDP-43
Cleavage

TDP-43 inclusions in patients suffering from ALS and FTLD-TDP contain both full length TDP-43 (FL-TDP-43) and TDP-43 C-terminal fragments of 25 kDa (CTF-25)(Neumann et al., 2006)(Igaz et al., 2008), but minimal or no N-terminal fragments (Kwong et al., 2014). Additionally, C-terminal fragments of 35 kDa (CTF-35) may also be found within inclusions in these patients (Zhang et al., 2007), but their presence is variable (Lee et al., 2011).

These CTFs are generated by the cleavage of TDP-43 by several different enzymes. The majority of studies have focused on caspase-3, which is a key executioner of apoptosis (Li et al., 2015) but is also involved in many cellular processes which do not lead to apoptosis (Shalini et al., 2014). Recent studies have provided evidence that calpain, which is activated by intracellular calcium ions, cleaves cytoplasmic TDP-43 \textit{in vivo}, as well (Yamashita et al., 2012)(Yang et al., 2014). Asparaginyl endopeptidase, the only known mammalian enzyme capable of cleaving C-terminal to asparagine residues, has also been shown to cleave TDP-43 \textit{in vivo} (Herskowitz et al., 2012). Notably, low levels of soluble, non-aggregated CTF-25 and CTF-35 are found in cells under control conditions (Dormann et al., 2009)(Nishimoto et al., 2010), and these fragments may be degraded by the proteasome (Bose et al., 2011)(I.-F. Wang et al., 2012)(Caccamo et al., 2015).
Phosphorylation

TDP-43 phosphorylation is used as a marker for pathological inclusion formation because phosphorylation and aggregation are highly correlated (Lee et al., 2011). FL-TDP-43 and CTFs may be phosphorylated by multiple kinases (Iguchi et al., 2012)(Liachko et al., 2014) at Ser379, Ser403, Ser404, Ser409, and Ser410 (Hasegawa et al., 2008). Experimental evidence strongly suggests that phosphorylation occurs in the cytoplasm after the conversion from detergent soluble to insoluble species (Nishimoto et al., 2010)(Dormann et al., 2009)(Budini et al., 2012). Although it has been shown that the phosphorylation of TDP-43 is neither necessary nor sufficient for inclusion formation (Dormann et al., 2009)(Zhang et al., 2009), its effects on the formation of TDP-43 oligomers and microaggregates are still unclear.

It has been reported in cell culture studies that phosphorylation of the 220-414 CTF increases aggregation and confers resistance to degradation (Zhang et al., 2010), and thus favors inclusion formation. Conversely, it has also been reported that phosphorylation decreases aggregation propensity (Brady et al., 2011)(Li et al., 2011), and so the phosphorylation of aggregates may actually be a protective mechanism meant to inhibit further aggregation (Li et al., 2011).

Additionally, it is unknown whether the phosphorylation of TDP-43 influences the protein’s function or toxicity. Experiments in C. elegans have shown that phosphorylation is required for TDP-43 neurotoxicity (Liachko et al., 2010)(Choksi et al., 2014). However, this effect on toxicity may be unique to the phosphorylation of human TDP-43 in C. elegans, as it has been shown in many other models that the
phosphorylation of TDP-43 or CTFs doesn’t affect toxicity (Zhang et al., 2009). Taken altogether, the evidence suggests that the earlier hypothesis of phosphorylation as a marker of insolubility but not a driver of aggregation or cell death (Dormann et al., 2009) is correct.

**Ubiquitination**

FL-TDP-43 and CTFs within cellular inclusions may be ubiquitinated (Neumann et al., 2006), but the specific TDP-43 residues that are tagged have not yet been described and no functional consequences of ubiquitination have been identified. Cytoplasmic TDP-43 has been shown to be ubiquitinated by the ubiquitin-conjugating enzyme UBE2E3 and deubiquitinated by ubiquitin isopeptidase Y (UBPY) (Hans et al., 2014), and aggregates may be tagged with K48- and K63-linked ubiquitin, ubiquilin-1 and -2, and p62 (Scotter et al., 2014). Studies focusing on the ubiquitination of TDP-43 all seemingly agree that, like phosphorylation, it is a late event that occurs in the cytoplasm and likely does not drive inclusion formation (Igaz et al., 2009)(Li et al., 2011)(Budini et al., 2012)(Farrawell et al., 2015).

**Nuclear Clearance**

Cells with TDP-43 cytoplasmic inclusions exhibit clearance of nuclear TDP-43 (Neumann et al., 2006). The consensus among researchers is that nuclear clearance occurs because cytoplasmic TDP-43 inclusions have the ability to bind and sequester free TDP-43 floating in the cytoplasm (Winton et al., 2008)(Yang et al., 2010)(Che et al.,
2011)(Budini et al., 2012)(Zhang et al., 2013)(Budini et al., 2015). Although predominantly found in the nucleus, TDP-43 moves rapidly and continuously between the nucleus and the cytoplasm (Ayala et al., 2008). The hypothesis is that because TDP-43 is highly prone to aggregation, over time more and more free TDP-43 binds to the inclusion as TDP-43 is naturally shuttling between the nucleus and the cytoplasm until no nuclear TDP-43 is left.

METHODS

Subjects

Forty seven subjects from the Boston University Chronic Traumatic Encephalopathy Center were utilized for this study. All subjects were former athletes, veterans, or civilians with a history of repetitive mild traumatic brain injury and were previously diagnosed with CTE. In order to select the subjects to be included in this study, first all subjects at the BU CTE Center diagnosed with CTE were identified. Second, all CTE-positive subjects examined before a certain time period where excluded due to a lack of sections immunostained for phosphoTDP-43. Next, all remaining CTE-positive subjects also diagnosed with FTLD, or with an “A” or “C” score greater than 1 according to the criteria set forth by the National Institute on Aging – Alzheimer’s Association (Montine et al., 2012) were excluded from this study. Finally, cases with numerous regions that were not available for examination were excluded from analysis but are shown in Appendix 1.
**Immunohistochemistry**

Human tissue was fixed in periodate-lysine-paraformaldehyde, and tissue blocks were paraffin-embedded and cut at 10 µm for immunohistochemistry. Sections were incubated at 4 °C overnight with antibodies to phosphoTDP-43 (pS409/410 mouse monoclonal; Cosmo Bio Co, Tokyo, Japan; 1:2000). Sections were counterstained with cresyl violet.

**Assessment of Neuropathological Features**

For each subject, the following seven regions were examined by the author along with a neuropathologist: substantia nigra, dorsolateral frontal cortex, rolandic cortex, anterior temporal cortex, amygdala, entorhinal cortex, and dentate gyrus. The severity of phosphoTDP-43 deposition was then graded on a 0 to 3 scale: a score of 0 indicated no TDP-43 inclusions on a section; a score of 1 indicated one to four TDP-43 inclusions on a section; a score of 2 indicated five to ten TDP-43 inclusions on a section; and a score of 3 indicated greater than ten TDP-43 inclusions on a section.

**Figure 3:** Regions of the brain examined. Adapted from Micklos, 2016.
Statistical Analysis

SPSS version 23.0 (IBM Inc., Chicago, IL, USA) was utilized for statistical analyses. Significance was set a priori at $\alpha = 0.05$. The Wilcoxon-Mann-Whitney $U$ Test was used to compare the means between different groups (CTE Stage, Age at Death, ...
Number of Playing Years, Age at First Exposure) based on TDP-43 presence (TDP- or TDP+). Logistic regression was performed to test for the effect of CTE Stage on TDP-43 presence independent of Age at Death. Finally, the Kruskal-Wallis H Test was used to compare the means between groups (Number of Effected Regions, Mean Severity of Affected Regions, CTE Stage) based on the TDP Staging Scheme developed in this study.

RESULTS

One hundred and thirty cases were identified as being previously diagnosed with CTE, and of those cases sixty one were identified as having a low likelihood of AD and also carrying no diagnosis of FTLD. Of these sixty one CTE-positive cases, fourteen were not able to be included in this study due to incomplete information. The demographic information of the final forty seven cases that were included is displayed in Table 1.
Table 1: Demographic Data

<table>
<thead>
<tr>
<th>CTE Stage</th>
<th>Number of Cases</th>
<th>Mean Age at Death</th>
<th>Mean Age at 1st Exposure</th>
<th>Mean Number of Playing Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>47</td>
<td>54.2 ± 18.8</td>
<td>10.9 ± 3.7</td>
<td>16.7 ± 7.2</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>43.8 ± 26.9</td>
<td>15.3 ± 2.5</td>
<td>18 ± 17</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>48.3 ± 17.7</td>
<td>8.9 ± 3.7</td>
<td>16.1 ± 6.3</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>59.1 ± 16.4</td>
<td>11.5 ± 3.3</td>
<td>17.5 ± 5.8</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>70.8 ± 7.6</td>
<td>12.5 ± 0.7</td>
<td>14 ± 6.2</td>
</tr>
</tbody>
</table>

Associations Between TDP-43 Inclusions, Age, and Exposure

Subjects with TDP-43 inclusions were older at death than subjects without inclusions. This does not come as a surprise, as the association between TDP-43 and age has been previously reported (Nakashima-Yasuda 2007)(Geser et al., 2010). Mean CTE Stage, a measure of the degree and distribution of pathological tau protein inclusions, was also significantly higher in subjects with TDP-43 inclusions. There was no statistically significant difference in the mean number of playing years, a rough estimate of the
number of mild traumatic brain injuries (mTBI) sustained, in subjects with CTE and TDP-43. These findings are summarized in Table 2:

**Table 2: TDP-43 inclusions are more frequent in older subjects and in advanced CTE**

<table>
<thead>
<tr>
<th></th>
<th>TDP –</th>
<th>TDP +</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cases</td>
<td>27</td>
<td>20</td>
<td>---</td>
</tr>
<tr>
<td>CTE Stage</td>
<td>2.2 ± 0.7</td>
<td>2.8 ± 0.9</td>
<td>0.016</td>
</tr>
<tr>
<td>Mean Age at Death</td>
<td>46.7 ± 16.6</td>
<td>64.1 ± 17.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean Age at 1st Exposure</td>
<td>9.8 ± 3.3</td>
<td>12 ± 3.9</td>
<td>0.086</td>
</tr>
<tr>
<td>Mean Number of Playing Years</td>
<td>15.6 ± 9.2</td>
<td>17.5 ± 5</td>
<td>0.493</td>
</tr>
</tbody>
</table>

**Age, But Not CTE Stage, Predicts Presence of TDP-43 Inclusions**

As shown above, subjects with TDP-43 inclusions also suffered from increased CTE Stage (increased tau pathology). However, as Age at Death varied significantly between TDP-43 groups and likely effects whether TDP-43 inclusions are present, logistic regression was performed to test whether CTE Stage had an effect on the presence of TDP-43 inclusions independent of age. It was found that while age did have an effect on the presence of TDP-43 inclusions ($\beta = 0.051$, $p = .019$), CTE Stage did not have a statistically significant effect on TDP-43 inclusions after controlling for age ($\beta =$
0.559, p = .228). Therefore, the presence of TDP-43 inclusions does not appear to be directly related to the overall severity of tau pathology within the brain.

**Staging TDP-43 Pathology in CTE**

TDP-43 pathology was identified in twenty out of forty seven (43%) CTE-positive cases without FLTD and with a low likelihood of AD, as shown in Figure 4:

**Figure 4:** TDP-43 pathology identified in nearly half of all CTE+, FTLD-, low-likelihood-of-AD subjects

![TDP-43 Positivity by Region](image)

Other studies have shown that in ALS (Brettschneider et al., 2013), AD (Josephs et al., 2014), and Behavioral Variant FTLD (Brettschneider et al., 2014), TDP-43 pathology may begin in particular regions and spread, leading to the development of the TDP-43-based staging schemes for these diseases shown in Table 3 below:
**Table 3:** Current TDP-43-based staging schemes. Adapted from Tan et al., 2015

<table>
<thead>
<tr>
<th></th>
<th>Amyotrophic lateral sclerosis</th>
<th>Behavioural variant frontotemporal dementia</th>
<th>Alzheimer's disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of stages</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Amygdala</td>
<td>Stage 3</td>
<td>Stage 1</td>
<td>Stage 1</td>
</tr>
<tr>
<td>Orbital cortex, gyrus rectus</td>
<td>Stage 3</td>
<td>Stage 1</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Inferior olive, paravascular red nucleus</td>
<td>Stage 2</td>
<td>Stage 2</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>Stage 4</td>
<td>Stage 2</td>
<td>Stage 2</td>
</tr>
<tr>
<td>Hippocampal dentate gyrus</td>
<td>Stage 4</td>
<td>Stage 2</td>
<td>Stage 3</td>
</tr>
<tr>
<td>Inferior temporal cortex</td>
<td>Stage 4</td>
<td>Stage 2</td>
<td>Stage 4</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>Stage 3</td>
<td>Stage 2</td>
<td>Stage 5</td>
</tr>
<tr>
<td>Hypoglossal nucleus, motor cortex</td>
<td>Stage 1</td>
<td>Stage 3</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Substantia nigra, locus coeruleus</td>
<td>Stage 2</td>
<td>Stage 4</td>
<td>Not assessed</td>
</tr>
</tbody>
</table>

In CTE, as shown in Appendix 1 and Appendix 2, there is no consistent initial focus of TDP-43 pathology and no clear stereotypical pattern of progression. Despite this, it is possible to accurately report the extent and severity of TDP-43 pathology in CTE with the staging scheme detailed in Table 4 below:

**Table 4:** TDP-43 Staging Scheme for CTE

**Stages of TDP-43 Pathology in CTE**

**Stage 0:** No TDP-43 inclusions in the substantia nigra, dorsolateral frontal cortex, or dentate gyrus

**Stage 1:** TDP-43 inclusions found in either the substantia nigra or the dorsolateral frontal cortex

**Stage 2:** TDP-43 inclusions found in the dentate gyrus or found in both the substantia nigra and the dorsolateral frontal cortex

**Stage 3:** TDP-43 inclusions found in the dentate gyrus, substantia nigra, and dorsolateral frontal cortex
**Figure 5**: Regions utilized in TDP-43 Staging Scheme for CTE. Adapted from Micklos, 2016.

The stages of this scheme exhibit a statistically significant difference between the mean number of effected regions. This scheme also reflects disease progression as measured by tau pathology (CTE Stage based on tau), and so an increase in TDP-43 Stage likely indicates a true worsening of disease. Finally, although a statistically significant difference between the mean severity of TDP-43 pathology within effected regions was not found, this is likely due to the small number of cases in each stage and the limited range of the TDP severity scoring used (0=none, 1=sparse, 2=moderate, 3=severe). These results are summarized in Table 5 below:
Table 5: TDP-43 Staging Scheme accurately reports both the extent and severity of TDP-43 pathology

<table>
<thead>
<tr>
<th>TDP Stage</th>
<th>n</th>
<th>Mean Number of Effected Regions per case (From 0 to 7)</th>
<th>Mean Severity of Effected Regions (Scored From 1 to 3)</th>
<th>Mean CTE Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDP Stage 0</td>
<td>28</td>
<td>0.1 ± 0.4</td>
<td>1 (n=1)</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>TDP Stage 1</td>
<td>7</td>
<td>1.7 ± 0.8</td>
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<td>.078, .057*</td>
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† for Kruskal-Wallis H Test
* Comparing Stages 1, 2, and 3 only

A full breakdown of how the cases in this study are staged according to this scheme is shown in Appendix 2. Of note is that one case (#41) was not given a TDP Stage nor included in the statistical analysis shown above in Table 4 because the dentate gyrus of that case was not examined. Also, one case (#4) which did exhibit TDP-43 pathology was given a TDP Stage of 0 according to this scheme. Case #4, however, was the only case out of twenty eight Stage 0 cases which had TDP-43 inclusions, and this case exhibited relatively minor TDP-43 pathology.

**TDP-43 and Activated Microglia in the Dorsolateral Frontal Cortex**

Lastly, a statistically significant association (p<.05) was found between the presence of TDP-43 inclusions and levels of activated microglia in the dorsolateral frontal cortex of CTE cases, as soon in Figure 6 below:
Figure 6: TDP-43 inclusions correlate with the presence of activated microglia

(Data on activated microglial cells in CTE cases kindly provided by Dr. Jonathan Cherry.)
DISCUSSION

Possible to Apply This TDP Staging Scheme to All FTLD- Populations?

Although this TDP staging scheme was developed in a specific cohort (CTE-positive, FTLD-negative, low-likelihood-of-AD), it may be reasonable to apply it to all FTLD-negative populations for two reasons:

1. Studies of Control Subjects Rarely Find TDP-43 Inclusions:

An extensive review of the literature identified six studies which reported on TDP-43 pathology found in cognitively normal subjects. These six studies are detailed in Appendix 3. All six studies examined the dentate gyrus and only one study identified TDP-43 inclusions in the dentate gyrus, and in only 12 of 323 cases. This same study examined a wide variety of regions and the TDP Staging Scheme developed in this paper mischaracterizes TDP-43 pathology in only 7 of the 323 cases (2.2%). Unfortunately the other five studies mainly focused on regions within the medial temporal lobe and so no definitive conclusions can be made on how often TDP-43 inclusions are found in the substantia nigra or the dorsolateral frontal cortex in control subjects.

2. TDP-43 Inclusions Are Found in the Dentate Gyrus and Dorsolateral Frontal Cortex Only in Later Stages of AD:

According to the TDP staging scheme for AD developed by Josephs et al., TDP-43 is not found in the dentate gyrus until AD TDP Stage III/V and is not found in the
dorsolateral frontal cortex until AD TDP Stage V/V (Josephs et al., 2014). An additional study by this group also reported that TDP-43 was found in the substantia nigra in almost half (24/51) of an AD TDP Stage IV and Stage V cohort (Jung et al., 2014). Unfortunately TDP-43 presence in the substantia nigra for Stage I-III cases was not reported, and so no conclusion can be made on which AD TDP stages inclusions may be found in the substantia nigra. However, assuming that inclusions are found in the substantia nigra only in AD TDP Stage III and beyond, the CTE TDP staging scheme developed in this study would accurately represent TDP-43 pathology even in the context of AD.

Why No Stereotypical Spreading Pattern of TDP-43 Pathology in CTE?

This study demonstrates that TDP-43 inclusions are clearly a feature of CTE, but fails to identify a stereotypical spreading pattern of TDP-43 pathology. In order to understand why this would be the case, one must consider the general conditions in which TDP-43 inclusions form and also how TDP-43 inclusions are likely developing specifically in CTE.

1. Acute Stress Causes a Shift in Localization and Cleavage of TDP-43

As stated previously, the significance of the ubiquitination, phosphorylation, cleavage, and nuclear clearance of TDP-43, the so-called “pathological changes” of TDP-43, is unclear. Many of these changes are seen during the cell’s normal response to stress, however, and it is very possible that these changes by themselves are innocuous
and not indicative of a pathological process. TDP-43’s response to oxidative, heat shock, and osmotic stress is as follows:

**Figure 7**: The normal response of TDP-43 to acute stress

A. A Portion of Nuclear TDP-43 Moves to the Cytoplasm

It is well-established that a portion of nuclear TDP-43 moves to the cytoplasm during the cell’s physiological response to stress (Colombrita et al., 2009)(Liu-Yesucevitz et al., 2010)(V. Ayala et al., 2011)(Dewey et al., 2011)(Iguchi et al., 2012)(McDonald et al., 2011)(Meyerowitz et al., 2011)(Bentmann et al., 2012)(Higashi et al., 2013)(Zhang et al., 2014)(Kabuta et al., 2015)(Kanazawa et al., 2015). Cell culture studies have shown that this nucleus-to-cytoplasm redistribution occurs in as little as 15 to 30 minutes upon application of stress (Dewey et al., 2011)(Zhang et al., 2014) and is
reversible upon removal of stress (McDonald et al., 2011)(Higashi et al., 2013)(Zhang et al., 2014). This shift is not unique to TDP-43, as many other RNA-binding proteins, such as hnRNP A1 (van der Houven van Oordt et al., 2000)(Allemand et al., 2005)(Lewis et al., 2007), hnRNP A2 (McDonald et al., 2011), hnRNP K (Habelhah et al., 2001), hnRNP Q (Quaresma et al., 2009), and RBM45 (Li et al., 2015) have also been shown to translocate to the cytoplasm in response to cellular stress.

Current evidence implicates the c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases (ERK), and p38 signaling cascades in the recruitment of TDP-43 to cytoplasmic stress granules upon application of stress (V. Ayala et al., 2011)(Meyerowitz et al., 2011)(Parker et al., 2012)(Higashi et al., 2013)(Moujalled 2013)(Suzuki et al., 2013). However, TDP-43 does not appear to be a substrate for any of the kinases involved in these pathways, so it is likely that cytosolic TDP-43 is bound either directly or to the same transcripts as other RNA-binding proteins that are modified in response to stress.

One very recent study has shown that hnRNP K always colocalizes with TDP-43 in stress granules and that recruitment of TDP-43 to stress granules is dependent upon phosphorylation of hnRNP K by cyclin-dependent kinase 2 (CDK2) (Moujalled et al., 2015). This would strongly suggest that TDP-43, which is rapidly shuttling in and out of the nucleus bound to mRNAs with other RNA-binding proteins such as hnRNP K attached, is recruited to SGs when other proteins bound to it or to the same mRNA transcripts are modified in response to stress.
B. Cytoplasmic TDP-43 May be Cleaved by Caspase-3 and/or Calpain

Cellular stressors that modify TDP-43 localization may lead to higher levels of CTFs in the cytoplasm (Iguchi et al., 2012)(Meyerowitz et al., 2011)(Higashi et al., 2013). This increase in CTFs is very likely due to caspase-3 activation caused by stress (Liu-Yesucevitz et al., 2010)(Dewey et al., 2011)(Higashi et al., 2013), although it has also been reported that low levels of stress which do not lead to caspase-3 activation may still lead to significant cleavage of TDP-43 (V. Ayala et al., 2011). Studies on the toxicity of CTFs have led to conflicting results and it is unclear whether CTFs are more (Zhang et al., 2009) or less (Voigt et al., 2010)(Suzuki et al., 2011) toxic than FL-TDP-43.

The CTFs created by TDP-43 cleavage are non-functional (Nonaka et al., 2009)(Zhang et al., 2009)(Nishimoto et al., 2010) for several reasons. First, both CTF-25 and CTF-35 lack a nuclear localization sequence and thus always remain in the cytoplasm. Second, CTF-25 lacks RRM1 and cannot bind RNA with the same efficiency as FL-TDP-43. Lastly, it is possible that TDP-43 normally functions as a homodimer and that the N-terminal is required for dimerization (Y.-T. Wang et al., 2013)(Budini et al., 2015), therefore both fragments lack the ability to dimerize.

It is unknown whether the cleavage of TDP-43 is unique among hnRNPs, as the cleavage of other RNA-binding proteins that shift to the cytoplasm due to stress does not appear to be an active area of investigation. Interestingly, other hnRNPs that interact with TDP-43 and shift localization in response to stress have been shown to be involved in the synthesis of X-linked inhibitor of apoptosis (XIAP) and B-cell lymphoma-extra
large (Bcl-xl), two major anti-apoptotic proteins that sequester active caspase-3 (XIAP) or prevent caspase activation (Bcl-xl). Upon phosphorylation, hnRNP A1 binds XIAP and Bcl-xl mRNA in the nucleus and shuttles both to the cytoplasm (Roy et al., 2014), however once in the cytosol bound hnRNP A1 also inhibits XIAP translation (Lewis et al., 2007)(Roy et al., 2014). HnRNP C1/C2 is a member of the internal ribosome entry site (IRES) complex for XIAP (Holcik et al., 2003), and thus is required for the translation of XIAP during stress. Finally, hnRNP K is either a transcription factor for XIAP or is involved in post-transcriptional modifications of XIAP mRNA (Xiao et al., 2013), and also promotes the splicing of Bcl-xl mRNA (Revil 2009). This involvement of TDP-43 binding partners with the production of the anti-apoptotic proteins XIAP and Bcl-xl raises the possibility that the cleavage of TDP-43 may be involved in negative regulation of activated caspase levels.

C. FL-TDP-43 and CTFs Are Cleared From the Cytoplasm

Upon removal of stress, it is unknown what fraction of cytoplasmic TDP-43 returns to the nucleus and what fraction is degraded. Studies have shown that the ubiquitin proteasome system (UPS), chaperone-mediated autophagy (CMA), and autophagy are all involved in the degradation of soluble FL-TDP-43 and CTFs (Caccamo et al., 2009)(Wang et al., 2010)(Huang et al., 2014)(Scotter et al., 2014). Experimental evidence indicates that CTFs are easier for the cell to degrade than FL-TDP-43, as it has been shown that CTF levels are more sensitive to inhibition or enhancement of either the ubiquitin proteasome system or autophagy (Wang et al., 2010)(Hans et al., 2014)(Huang
et al., 2014) and that CTFs have a significantly shorter half-life than FL-TDP-43 (Pesiridis et al., 2011)(Hans et al., 2014)(Huang et al., 2014). This has led to the hypothesis that cleavage of the protein is a favorable event which enhances TDP-43 degradation (Huang et al., 2014).

Inhibition of the ubiquitin proteasome system, but not autophagy, has been shown to lead to the formation of TDP-43 aggregates, and so the ubiquitin proteasome system enhancement has been proposed as a therapeutic target for TDP-43 proteinopathies. Finally, the cell under normal conditions has the ability to clear aggregates that form by utilizing a combination of the two systems, with the ubiquitin proteasome system degrading soluble TDP species and autophagy clearing oligomers, microaggregates, and macroaggregates (Scotter et al., 2014).

2. TDP-43 Inclusions Are a Consequence of Chronic Stress

Figure 8: Chronic stress results in TDP-43 inclusions

- 1. Normal State
- 2. Add Mild Stress
- 3. TDP-43 Cleaved into Fragments
- 4. Fragments Aggregate, Proteasome and Nuclear Import Impaired?
- 5. Aggregate Sequesters FL-TDP-43
- 6. Aggregate Phosphorylated and Ubiquitinated
- 7. Nuclear Clearance of TDP-43, Induces Mitophagy and Sequesters Other Proteins?
- 8. Cell Degenerates

Key:
- TDP-43
- TDP-43 Fragments
- Crossed-5-Carboxyl, etc
- Proteasome
- Transcription Factors, Other Proteins
It is notable that models of acute stress result in a transient increase in cytoplasmic TDP-43 and CTFs, but not in the formation of permanent TDP-43 inclusions (Colombrita et al., 2009)(Sato et al., 2009)(Dewey et al., 2011)(Iguchi et al., 2012)(McDonald et al., 2011)(Meyerowitz et al., 2011)(Higashi et al., 2013)(Zhang et al., 2014). If the partial redistribution of TDP-43 from nucleus to cytoplasm is part of an intrinsic response meant to help the cell cope with acute stress, it would follow that this response shouldn’t result in the formation of pathological inclusions.

Inclusions are formed, however, in models overexpressing cytoplasmic CTFs (Caccamo et al., 2009)(Nonaka et al., 2009)(Ash et al., 2010)(Che et al., 2011)(Yamashita et al., 2014)(X. Wang et al., 2015), in models with ubiquitin proteasome system inhibition (Wang et al., 2010)(Tashiro et al., 2012)(Scotter et al., 2014), and in models with inhibition of nuclear import (Winton et al., 2008)(Nishimura et al., 2010). Experimental evidence indicates that application of sub-lethal cellular stress over a period of weeks or months would lead to not just one of these conditions but all three, and it is very likely that TDP-43 inclusions are formed over time through their combined efforts.

Two recent mouse models have provided evidence that chronic, sub-lethal stress results in chronically elevated levels of FL-TDP-43 and/or CTFs in the cytoplasm, and thus favor inclusion formation. The first study demonstrated that lipopolysaccharide (LPS) treatment, which is known to activate astrocytes and microglia, for a period of two months resulted in a sustained increase in both the cytoplasmic-to-nucleus TDP-43 ratio and in levels of insoluble TDP-43 species (Correia et al., 2015). The second study
showed that rTBI, which causes long-lasting neuroinflammation (Shitaka et al., 2011) (Aungst et al., 2014), resulted in increased levels of FL-TDP-43 thirty days after the initial injury (Zhang et al., 2015).

Other studies have also provided evidence that chronic sub-lethal stress conditions that cause a shift in TDP-43 localization would result in the inhibition of autophagy and UPS by several different mechanisms. Elevated levels of cytoplasmic CTFs have been shown to decrease autophagy and UPS function in a toxic gain-of-function manner (Caccamo et al., 2015). TDP-43 knockdown models have been shown to exhibit decreased mRNA and protein levels of autophagy-related 7 (ATG7), a protein necessary for autophagy, as well as autophagy inhibition (Bose et al., 2011). Also, loss of nuclear TDP-43 results in the expression of cryptic exons in the ATG4B mRNA transcript, another protein necessary for autophagy, which disrupt its translation and promotes nonsense-mediated decay of the transcript (Ling et al., 2015).

Lastly, chronic sub-lethal stress conditions that result in a shift in TDP-43 localization would very likely result in general impairment of nuclear import. TDP knockdown leads to decreased Ran levels (Sephton et al., 2011) (Ling et al., 2015), which is necessary for the nuclear import of many proteins, including TDP-43 itself (Ward et al., 2014).

3. Persistent Stress Granules vs. Separate Inclusions Seeded by CTFs

Researchers are currently debating whether TDP-43 inclusions are a result of stress granules that fail to disassemble or are separate inclusions, possibly seeded by
CTFs. Post-mortem studies on TDP-43 inclusions in ALS and FTLD-TDP tissue have yielded conflicting results. Some groups have reported co-localization of TDP-43 inclusions with SG markers (Volkening et al., 2009) (Liu-Yesucevitz 2010), while others have reported no SG markers within TDP-43 inclusions (Colombrita et al., 2009). It is also very possible that SG markers are not part of the original inclusions but are later recruited as the inclusion mature (McGurk et al., 2014), as SG proteins are inherently prone to aggregation (Aulas et al., 2015).

Further muddying the waters is the fact that a distinction may need to be drawn between TDP-43 inclusions found within the brain and inclusions found in the spinal cord. Just as ALS and FTLD-TDP inclusions within the brain consist of mostly CTFs and spinal cord inclusions consist of mostly FL-TDP-43 (Igaz et al., 2008), the presence of SG markers may depend on whether one is examining the brain or the spinal cord. Along these lines, one study has reported finding SG markers in spinal cord TDP-43 inclusions but not within brain inclusions (Bentmann et al., 2012).

Cell culture studies have provided little evidence to settle the debate, largely due to the heterogeneity of cell types and stressors used. Different cell types respond to the same stressors in different ways, and different stressors applied to the same cell types result in different stress granule dynamics (Aulas et al., 2015). Notable, however, is the fact that studies have reported TDP-43 to be found in small cytoplasmic granules, and not SGs, upon exposure to an endogenous oxidative stressor (as opposed to the artificial stressors used in most studies) (Kabuta et al., 2015), and also stress at higher levels (Zhang et al., 2014) or of longer periods (Higashi et al., 2013).
4. Pro-inflammatory Cytokines Likely Drive the Shift in Localization and Then Cleavage of TDP-43 Seen During the Acute Response to Injury in Animal Models

Recent mouse and rat models have shown that a single TBI induces a delayed stress-response shift from the nucleus to the cytoplasm and then cleavage of TDP-43 (Yang et al., 2014)(H. Wang et al., 2015), and notably didn’t result in the formation of TDP-43 cytoplasmic inclusions. However, both models demonstrated a response very similar to that seen in mouse models of other injuries, such as axotomy (Moisse, Mepham, et al., 2009)(Moisse, Volkening, et al., 2009) and axonal ligation (Sato et al., 2009). First, cytoplasmic TDP-43 levels are largely unchanged at 24 hours post-injury, then increases begin to reach significance on Day 3 post-injury, followed by cytoplasmic TDP-43 levels peaking at Day 7 post-injury, and then finally normalizing by Day 14 (Moisse, Volkening, et al., 2009)(H.-K. Wang et al., 2015) or Day 28 (Sato et al., 2009) post-injury. This pattern strongly suggests that TDP-43 inclusion formation does not occur acutely following injury. More importantly, it also suggests that the shift to the cytoplasm and then cleavage of TDP-43 is not an immediate event that occurs within minutes of the primary injury, but is instead due to a common secondary response to injury.

That shared secondary response is very likely the neuroinflammatory response to injury. Models of TBI (d’Avila et al., 2012)(G. Wang et al., 2013)(Y. Wang et al., 2014) and axotomy (Moisse, Volkening, et al 2009)(Villacampa et al., 2015) have shown that markers of activated microglia, such as Iba-1 and TSPO, exhibit a rise and fall in response to injury which mimics that of cytoplasmic TDP-43 and CTFs: activated
microglia levels in the injured area are largely unchanged at 24 hours post-injury, then increases begin to reach significance on Day 3 post-injury, followed by levels peaking around Day 7 post-injury, and then steadily decreasing until near-normal levels are reached by Day 28 post-injury.

Additionally, the polarization of microglia has also been shown to follow this pattern in mouse models of spinal cord injury (Kigerl et al., 2009) and TBI (G. Wang et al., 2013). Anti-inflammatory M2 macrophages are initially increased and may predominate early, but they return to baseline levels by Day 7 post-injury. Pro-inflammatory M1 macrophages, however, are increased starting on Day 3, levels peak around Day 7 post-injury, and remain elevated at least 14 days after injury.

The question then becomes which particular aspect of neuroinflammation is responsible for the shift in localization and then cleavage of TDP-43? Although activated microglia are known to release reactive oxygen species (ROS) that cause oxidative stress (Hu et al., 2015) and may stress neurons via other mechanisms, it is likely that the pro-inflammatory cytokine milieu which leads to M1 microglial activation is also driving the initial changes observed in TDP-43.

One very recent study has demonstrated that TNF-α has the ability to induce the shift of TDP-43 from the nucleus to the cytoplasm (Correia et al., 2015). This is not surprising as TNF-α is known to activate the JNK and p38 signaling pathways (Wajant et al., 2003), which as previously mentioned are thought to be responsible for the cytoplasmic accumulation of TDP-43. The interleukin-1 family (IL-1) activates the JNK
and p38 signaling pathways as well (Weber et al., 2010), and so IL-1α and/or IL-β may also be involved in the cytoplasmic accumulation of TDP-43.

**From Repetitive mTBI to Chronic Neuroinflammation and then TDP-43 Cellular Inclusions**

The following is a proposed scheme of how rmTBI may be leading to the TDP-43 inclusions found in cases of CTE:

1. RmTBI causes chronic neuroinflammation

2. Chronic neuroinflammation causes chronically elevated levels of cytoplasmic TDP-43 and CTFs

3. CTFs aggregate with each other and then FL-TDP-43 to create inclusions and cause TDP-43 nuclear clearance

**1. rmTBI Causes Chronic Neuroinflammation**

Studies of mice (Loane et al. 2014) and humans (Ramlackhansingh et al., 2011)(Smith et al., 2013) have reported that a single TBI causes neuroinflammation that may persist for months or even years. Additionally, rTBI induces long-lasting neuroinflammation in mouse models (Shitaka et al., 2011)(Aungst et al., 2014)(Petraglia et al., 2014) and there is widespread agreement among researchers that rTBI may cause chronic neuroinflammation in humans (Coughlin et al., 2015), as well.
2. Chronic Neuroinflammation Causes Chronically Elevated Levels of Cytoplasmic TDP-43 and CTFs

Chronically activated microglia secrete pro-inflammatory cytokines such as TNF-α and IL-1β, and studies have shown that pro-inflammatory cytokine levels may remain elevated for up to a year after even a single TBI (Kumar et al., 2014). As mentioned previously, chronic stress results in a sustained increase in cytoplasmic TDP-43 CTFs. Activation of astrocytes and microglia with LPS treatment for two months has been shown to result in a sustained increase in cytoplasmic TDP-43 (Correia et al., 2015) and rTBI has also directly been shown to lead to increased levels of cytoplasmic TDP-43 thirty days after the initial injury (Zhang et al., 2015).

3. CTFs Aggregate with Each Other and then FL-TDP-43 to Create Inclusions

CTFs are very aggregate-prone, even more so than FL-TDP-43 (Igaz et al., 2009)(Zhang et al., 2009)(Nonaka et al., 2009), and numerous studies have demonstrated that when cytoplasmic CTF levels are increased they form aggregates (Caccamo et al., 2009)(Nonaka et al., 2009)(Ash et al., 2010)(Che et al., 2011)(Yamashita et al., 2014)(X. Wang et al., 2015). Early aggregates are likely cleared by the combined work of UPS and autophagy, but a sustained reduction of nuclear TDP-43 would lead to the impairment of these systems and also impaired nuclear import (Bose et al., 2011)(Sephton et al., 2011)(Ling et al., 2015). Over time these inefficiencies would likely result in CTF aggregates which are not degraded, and these aggregates would have the ability to recruit FL-TDP-43 and cause nuclear clearance (Winton et al., 2008)(Yang...
The Need for Further Study of the Relationship Between rmTBI, Inflammation, and TDP-43 Inclusions

The theory that chronic neuroinflammation drives TDP-43 inclusion formation is bolstered by the finding of this study that levels of activated microglia correlate with the presence of TDP-43 inclusions in the dorsolateral frontal cortex. However, as TDP-43 has previously been shown to induce inflammation (Herman et al., 2012) and other pathologies are likely present, further research on the relationship between neuroinflammation and TDP-43 inclusions in CTE is sorely needed.

Although inflammation in CTE has not yet been systematically studied, it is likely that some regions of the brain are more prone to inflammation upon rmTBI than others and also that inflammation hotspots may vary between subjects. It could be that some subjects exhibit more inflammation in the substantia nigra, while others exhibit more inflammation in the neocortex or the medial temporal lobe. If pro-inflammatory cytokines do indeed drive the formation of TDP-43 inclusions, this variation in inflammation would explain the pattern of TDP-43 pathology found in CTE.
APPENDIX 1

All Cases Sorted According to Present CTE Staging Scheme Based on Tau

1=TDP-43 present, 0=TDP-43 not present, UN = unexamined or unknown

Cases in red were not included in this study because they contained more than two unexamined regions or were not given a CTE Stage

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APPENDIX 2

All Studied Cases Sorted According to Proposed TDP Staging Scheme for CTE

0=No TDP-43, 1=1-4 TDP-43 inclusions, 2=5-10 TDP-43 inclusions, 3=Greater than 10 TDP-43 inclusions, UN = unexamined or unknown

Case 41 not given a TDP Stage because dentate gyrus not examined

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## APPENDIX 3

### Studies of TDP-43 Pathology in Cognitively Normal Subjects

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<th>TDP- Mean Age at Death</th>
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<td>Nakashima-Yasuda et al., 2007</td>
<td>1/33</td>
<td>98</td>
<td>75 ±12</td>
<td>Only medial temporal lobe examined. 1 case with TDP found in CA1.</td>
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<td>Wilson et al., 2011</td>
<td>2/63</td>
<td>82</td>
<td>71</td>
<td>Examined amygdala initially in all cases, both TDP+ cases then examined in frontal cortex, superior temporal cortex, cingulate cortex, anterior and posterior hippocampus, striatum, midbrain, pons, medulla, and spinal cord. Concluded TDP in amygdala only.</td>
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<td>Arnold et al., 2013</td>
<td>40/110</td>
<td>88 ±6</td>
<td>86 ±6</td>
<td>Only medial temporal lobe examined in all cases. All 40 cases with TDP in either amygdala, parahippocampal gyrus, and/or subiculum.</td>
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<td>Kovacs et al., 2013</td>
<td>4/51</td>
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<td>Specific TDP+ regions not reported</td>
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<td>Nascimento et al., 2015</td>
<td>34/323</td>
<td>74 ±13</td>
<td>69 ±11</td>
<td>Initially examined amygdala, hippocampal formation, and inferior/middle temporal gyrus only. If TDP+, then also examined anterior cingulate gyrus, superior frontal gyrus, and superior colliculus.</td>
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<td>- Of 12 cases TDP+ in dentate gyrus: 1 case with no other regions TDP+, 4 cases with 1 other TDP+ region.</td>
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<td>- 2 of 6 cases with &gt;3 TDP+ regions are TDP- in dentate gyrus.</td>
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<td><strong>TDP Staging Scheme mischaracterizes extent and severity of TDP-43 pathology in 7/323 cases (2.2%).</strong></td>
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<td>Uchino et al., 2015</td>
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<td>81 ±9</td>
<td>77 ±10</td>
<td>Examined anterior hippocampus, amygdala, medulla, and lumbar spinal cord. TDP found most often in uncus, also found in inferior olivary complex and spinal cord.</td>
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REFERENCES


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Dormann, Dorothee, Anja Capell, Aaron M. Carlson, Sunita S. Shankaran, Ramona Rodde, Manuela Neumann, Elisabeth Kremmer, et al. “Proteolytic Processing of


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Kawahara, Yukio, and Ai Mieda-Sato. “TDP-43 Promotes microRNA Biogenesis as a Component of the Drosha and Dicer Complexes.” Proceedings of the National


Moisse, Katie, Jennifer Mepham, Kathryn Volkening, Ian Welch, Tracy Hill, and Michael J. Strong. “Cytosolic TDP-43 Expression Following Axotomy Is Associated with Caspase 3 Activation in NFL−/− Mice: Support for a Role for


CURRICULUM VITAE

Douglas Barnes
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(978) 436-1861
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Year of Birth: 1986

EDUCATION

Boston University School of Medicine, Boston, MA
Masters in Medical Sciences – expected graduation in May 2016

Boston College. Class of 2009 Chestnut Hill, MA
Bachelor of Science in Biology

Boston University Emergency Medical Services, July 2008 Boston, MA
Summer 2008 EMT-B Course, Earned MA EMT-B certification

Bates College, 2006 Lewiston, ME
Freshman Year

Concord Carlisle High School. June 2005 Concord, MA
Captain, Varsity Men’s Alpine Ski Team

PROFESSIONAL EXPERIENCE

Research Assistant May 2015 – December 2015 Bedford, MA
Boston University Chronic Traumatic Encephalopathy Center
- Studied pathological TDP-43 protein inclusions in CTE
- Assisted in the collection of frozen and fixed brain tissue samples at the Edith Nourse Rogers Memorial Veterans Hospital

Infantry Squad Leader 82nd Airborne Division, March 2013 – August 2014
- Responsible for the fitness, training, control, and actions of the nine soldiers in my squad
- Attended the Infantry Advanced Leader Course

Infantry Team Leader 82nd Airborne Division, January 2012 – February 2013
- Directly responsible for the control and training of the three soldiers in my team
- Deployed to Afghanistan in 2012. Promoted to Noncommissioned Officer in recognition of demonstrated leadership.
Infantry Riflemen 82nd Airborne Division, May 2010 – December 2012
- Completed the Defense Language Institute 6 month course on Chinese-Mandarin

Manager: A-Team Painting, May 2006 – September 2007 Concord, MA
Worked in a partnership with 2 others running our own business
- Developed job opportunities
- Provided estimates for potential clients
- Managed the finances of the company

VOLUNTEER EXPERIENCE
Cape Fear Valley Hospital September 2012 – February 2013 Fayeteville, NC
- Assisted with processing patients into the Emergency Department
- Accumulated approximately 110 hours of experience