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Quantification of Phosphorylated TDP-43 Protein in Concomitant Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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April 12th, 2015

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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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The appearance of frontotemporal dementia (FTD) in a subset of amyotrophic lateral sclerosis (ALS) patients has prompted researchers to investigate pathological and genetic overlaps between the two diseases. The aim of this study was to determine if there is a link between the pathological burden of pTDP-43 protein and the presence of dementia in ALS patients. Quantitative analysis was performed on cognitively normal ALS patients, ALS patients with dementia, and patients with frontotemporal lobar degeneration (FTLD). Our results show that demented ALS patients tend to have a higher burden of pTDP-43 pathology in the dentate gyrus than do cognitively normal ALS patients. However, in four other regions there was no difference in pTDP-43 pathology between the subject groups. Also, two ALS patients had high levels of pTDP-43 in the dentate gyrus but did not present with FTD symptoms. This provides some insight as to the correlation between this protein and clinical dementia, though the exact relationship is not yet certain.

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My final thanks is reserved for Jonathan Glass, M.D., my mentor, advisor, and principal investigator. Dr. Glass has been one of the most influential people in my growth as a student, researcher, aspiring physician, and scientist. His patience and guidance were instrumental in the writing of this thesis, but his curiosity and appetite for science served to inspire and fuel me to work through frustration and tedium.

* * *

This thesis is dedicated to my grandfather, John Gholson Kittredge, who passed in 2013 as a result of neurodegenerative disease.

Table of Contents

Abstract

The appearance of frontotemporal dementia (FTD) in a subset of amyotrophic lateral sclerosis (ALS) patients has prompted researchers to investigate pathological and genetic overlaps between the two diseases. The aim of this study was to determine if there is a link between the pathological burden of pTDP-43 protein and the presence of dementia in ALS patients. Quantitative analysis was performed on cognitively normal ALS patients, ALS patients with dementia, and patients with frontotemporal lobar degeneration (FTLD). Our results show that demented ALS patients tend to have a higher burden of pTDP-43 pathology in the dentate gyrus than do cognitively normal ALS patients. However, in four other regions there was no difference in pTDP-43 pathology between the subject groups. Also, two ALS patients had high levels of pTDP-43 in the dentate gyrus but did not present with FTD symptoms. This provides some insight as to the correlation between this protein and clinical dementia, though the exact relationship is not yet certain.

Background

Overview

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing and fatal motor neuron disease. Frontotemporal dementia (FTD) is a similarly harmful disease that is associated with cognitive and behavioral dysfunction. Though these two diseases are clinically very different, some ALS patients show signs of FTD. Our question was: why do some ALS patients have dementia while others do not? Given that accumulation of phosphorylated transactive response DNA binding protein 43 is a pathological hallmark of both diseases (Arai & Masato, 2006), we aimed to find if there is a correlation between the distribution of this protein in the brain and the clinical feature of FTD in ALS patients.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a devastating yet poorly understood neurodegenerative disease that is characterized by the progressive loss of voluntary movement associated with rapidly progressing muscle atrophy, weakness, and spasticity(Wijesekera & Leigh, 2009). The prevalence and incidence of ALS is approximately 5 and 1-2 per 100,000 per year, respectively. Males appear to be more commonly diagnosed with ALS than women at a ratio of 1.5:1, and age is another risk factor with a median onset of 60 years old for sporadic cases. Roughly 10% of all ALS cases are familial, and the remaining 90% of cases do not demonstrate a family history (though some carry disease-causing mutations); these cases are referred to as sporadic ALS (Chen et al., 2013).

The location of the onset of the symptomatic muscle weakness and atrophy varies and can include the upper limbs, lower limbs, or bulbar muscles (Wijesekera & Leigh, 2009). Difficulty speaking, breathing, and swallowing in bulbar-onset patients as well as weakness and atrophy in the musculature of limb-onset patients are the result of the selective degeneration of upper and lower motor neurons that control voluntary movements (Appel et al., 2011). ALS spares sensory systems and oculomotor control. Deaths associated with ALS result from respiratory failure as the diaphragm weakens (Pasinelli & Brown, 2006). The majority of ALS patients typically die within 3-5 years of diagnosis, though a minority of patients progress at a relatively slower rate. ALS currently does not have a cure, and the only FDA approved drug for ALS, Riluzole, extends the prognosis by approximately 3 months (Chen et al., 2013).

Familial ALS

Familial ALS (fALS) is clinically indistinguishable from sporadic ALS (sALS) with the exception of a later age of onset for sALS cases (Chen et al., 2013). An estimated 20% of familial ALS (fALS) cases are caused by a mutation in the superoxide dismutase 1 (SOD1) gene, and over 30% of fALS cases are the result of mutations in the chromosome 9 open reading frame 72(C9ORF72) gene. The remainder of fALS cases are caused by rare mutations in vesicle associated membrane protein B (VAPB), angiogenin, alsin, senataxin, and genes that code for fused in sarcoma (FUS)

and the 43-kDa TARDNA-binding protein (TDP-43), or by mutation in genes yet to be identified.

Frontotemporal Dementia

Frontotemporal dementia (FTD) is a broad term for a heterogeneous group of clinical dementias that represent up to 20% of cases of presenile dementia and are characterized by language dysfunction, behavioral dysfunction, or both (Cairns, Neumann, & Bigio, 2007). The incidence of FTD is approximately 2.7-4.1 per 100,000 per year with a prevalence of 15-22 per 100,000. Unlike ALS, gender does not appear to be a risk factor for FTD (Onyike & Diehl-Schmid, 2013). This disease can be either familial (50%) or sporadic, yet there are no clinically distinguishing features between the two forms (Verma, 2014). FTD can be classified as behavioral variant FTD (bvFTD), semantic dementia, or progressive nonfluent aphasia. The most common form of FTD is bvFTD, which accounts for an estimated two-thirds of FTD cases and is the most common form of dementia seen in patients with ALS. Behavioral variant FTD patients tend to show behavioral disinhibition, marked changes in personality, signs of apathy, emotional distancing, hyperorality, and stereotyped behavior with relative sparing of both visuospatial capabilities and episodic memory (Van Langenhove et al., 2012).

Frontotemporal Lobar Degeneration

Frontotemporal Lobar Degeneration (FTLD) is one of the 3 most common neuropathological diagnoses underlying the clinical diagnosis of dementia alongside Alzheimer's disease (AD) and dementia with Lewy bodies (LBD) (Neumann, 2009). FTLD is characterized by atrophy in the frontal and temporal cortices as well as the hippocampus and caudate nucleus, and is considered to be the underlying pathological diagnosis in patients with frontotemporal dementia. The neuronal loss and gliosis is typically most evident in the frontal and temporal neocortex. FTLD can be subcategorized based on specific neuropathological features. The first of these is FTLD with tau inclusions or FTLD-tau, which can be further categorized into Pick's disease or FTLD-tau (PiD), corticobasal degeneration or FTLD-tau (CBD), and progressive supranuclear palsy or FTLD-tau (PSP) (Bigio, 2013). FTLD-tau accounts for approximately 40% of FTLD cases (Neumann, 2009). The second main diagnosis is FTLD with ubiquitin and p62 inclusions or FTLD-U, which encompasses the more common FTLD-U with TDP-43 inclusions (FTLD-TDP), FTLD-U with fused in sarcoma inclusions (FTLD-FUS), FTLD-U without tau, TDP-43, or FUS inclusions (FTLD-UPS), and FTLD without inclusions (i.e. tau, TDP-43, FUS, ubiqituin, or p62) (FTLD-ni) (Bigio, 2013). FTLD-U accounts for about 60% of all FTLD cases (Neumann, 2009).

Diagnosing FTLD

Bigio (2013) suggests that FTLD may be diagnosed post-mortem after histological inspection of the following 13 sections of the brain: "…middle frontal gyrus;

superior and middle temporal gyri; inferior parietal lobule; occipital cortex; anterior cingulate gyrus; amygdala; hippocampus with dentate gyrus and entorhinal cortex; basal ganglia at the anterior commissure with the caudate putamen, globus pallidus, and the nucleus basalis of Meynert; thalamus with subthalamic nucleus; cerebellar cortex and dentate nucleus; midbrain with substantia nigra; pons with locus coeruleus; and medulla with dorsal motor nucleus of the vagus and hypoglossal nucleus (Bigio 2013)." Using hematoxylin-eosin (H&E) staining, the sections should be evaluated for neuronal loss, sclerosis, vascular injury, gliosis, and microvascular lesions according to criteria as specified by the National Institute on Aging-Alzheimer's Association (NIA-AA) (Hyman et al., 2012). These sections should also be evaluated for AD and LBD pathology, and any sections or stains that imply non-AD and non-LBD pathology are grounds for FTLD classification. Immunostaining for tau, ubiquitin, p62, FUS, and TDP-43 should be performed on the available sections in order to classify the type of FTLD pathology. Immunostains positive for tau indicate a diagnosis of FTLD-tau and immunostains positive for ubiquitin or p62 indicate a diagnosis of FTLD-U. Ubiquitin- or p62-positivity accompanied by positivity to the FUS antibody indicates a further classification of FTLD-FUS, while ubiquitin- or p62-positivity accompanied by TDP-43-positive staining indicates FTLD-TDP (Bigio, 2013).

TDP-43

The 43 kDa transactive response DNA binding protein (TDP-43) plays a role in numerous steps of the regulation of gene expression, such as the splicing and

transport of RNA (Janssens & Van Broeckhoven, 2013). TDP-43 is found mainly in the nucleus, though it is known to shuttle between the nucleus and cytosol. When hyper-phosphorylated, TDP-43 cytosolic inclusions often appear in patients (Huang, Lin, & He, 2013). The accumulation and aggregation of the phosphorylated form of TDP-43 (pTDP-43) has been identified in ubiquitin-positive neuronal and glial inclusions in patients with ALS, FTD, and in patients with both ALS and FTD (Arai & Masato, 2006). Though the exact role of pTDP-43 in these diseases is unknown, researchers have speculated that TDP-43 aggregates themselves are toxic, either due to a gain of toxic function or a loss of normal TDP-43 function (Gendron, Josephs, & Petrucelli, 2010).

FTLD-TDP

FTLD-TDP cases typically demonstrate a variety of immunoreactive TDP lesions such as neuronal cytoplasmic inclusions, neuronal intranuclear inclusions, dystrophic neurites, and oligodendroglial inclusions(Armstrong, Carter, & Carins, 2012). The neuronal cytoplasmic inclusions in FTLD-TDP tend to be round or spike-like in shape, while oligodendroglial inclusions are coiled. Neuronal intranuclear TDP inclusions are circular, lenticular, or spindle-shaped, and dystrophic neurites are more elongated.

FTD-ALS Spectrum

Current research points toward a disease spectrum composed of FTD and ALS at opposite ends of the continuum (Van Langenhove et al., 2012). Approximately 15%

of patients with FTD also meet the criteria for a diagnosis of ALS. Between 15%-18% of ALS patients also meet the criteria for FTD. Patients who present with both ALS and FTD show cognitive changes consistent with bvFTD. The proposition of an FTD-ALS spectrum is supported by ALS patients who do not meet diagnostic criteria for dementia, yet show abnormalities in executive functions such as planning, inhibiting behavior, and problem solving – all of which are associated with frontal lobe dysfunction. Reports regarding the proportion of ALS patients with abnormal executive function vary, with estimations ranging from 22% to 36% (Van Langenhove, Van der Zee, & Van Broeckhoven, 2012, Verma, 2014).

C9orf72

A hexanucleotide(GGGGCC) repeat expansion at the C9orf72 gene on chromosome 9p21 is believed to be responsible for up to 50% of familial ALS or familial FTD (Verma, 2014). In patients with this G_4C_2 expansion, over 95% have either ALS, bvFTD, or a combination of the two diseases. In addition, expansion mutations in the C9orf72 gene have been linked to around 5% of sporadic cases of FTD

Project Goals

The primary goal of this research was to explore a potential pathological correlation between ALS and FTD. We asked: does the pTDP-43 pathology in ALS, FTD, and ALS+FTD patients lend any insight into why some patients with ALS also have FTD? We quantitatively analyzed the presence and distribution of pTDP-43 inclusions in post-mortem tissue of three population groups: demented ALS patients, cognitively

normal ALS patients, and demented patients diagnosed with FTLD-TDP in the absence of ALS. Using pre-collected clinical data to describe the subject groups, we focused on comparing the pTDP-43 quantity and distribution in select regions of the brain associated with the cognitive dysfunction seen in bvFTD under the hypothesis that demented patients would have higher quantities of pTDP-43 in these regions than non-demented patients.

Materials and Methods

Subject Population

Brain tissues were analyzed from three different subject groups: ALS without dementia, ALS with clinical dementia, and demented patients without ALS who had an FTLD-TDP diagnosis. The age at onset, age at death, and disease characteristics for all three patient populations can be seen in Table 1 below. All cases except for five FTLD patients (from between 1986 and 2000) were taken from autopsies at the Emory Center for Neurodegenerative Disease since 2004.

Characteristic	Total (n=46)	ALS (n=20)	ALS+FTD (n=9)	FTLD (n=17)	Statistical Value[a]	P Value
Age at onset. mean (SD), years	59.6 (9.0)	58.2 (10.5)	60.1 (7.8)	60.9 (7.8)	0.9821	0.8028
Age at death, mean (SD), years	64.5 (9.1)	62.7 (10.1)	64.5 (8.2)	66.9 (8.4)	1.780	0.6194
Disease duration, mean (SD), years	4.03 (2.1)	3.84 (2.2)	4.40 (2.1)	7.27 (4.2)	7.962	0.0187*

Table 1 – Disease characteristics for the three subject groups. [a]The statistical test used to evaluate differences in the means between subject groups was a one-way non-parametric ANOVA, with the Kruskal-Wallis test statistic value shown. *Significance was evaluated at the P <0.05 level. The statistical significance found in disease duration was followed up with 3 pairwise comparisons via Mann-Whitney non-parametric t-tests. The *P*-values were as follows: ALSvsALS+FTD – *P*=0.3761, ALS+FTDvsFTD – *P*=0.0864, and ALSvsFTD – *P*=0.0057.

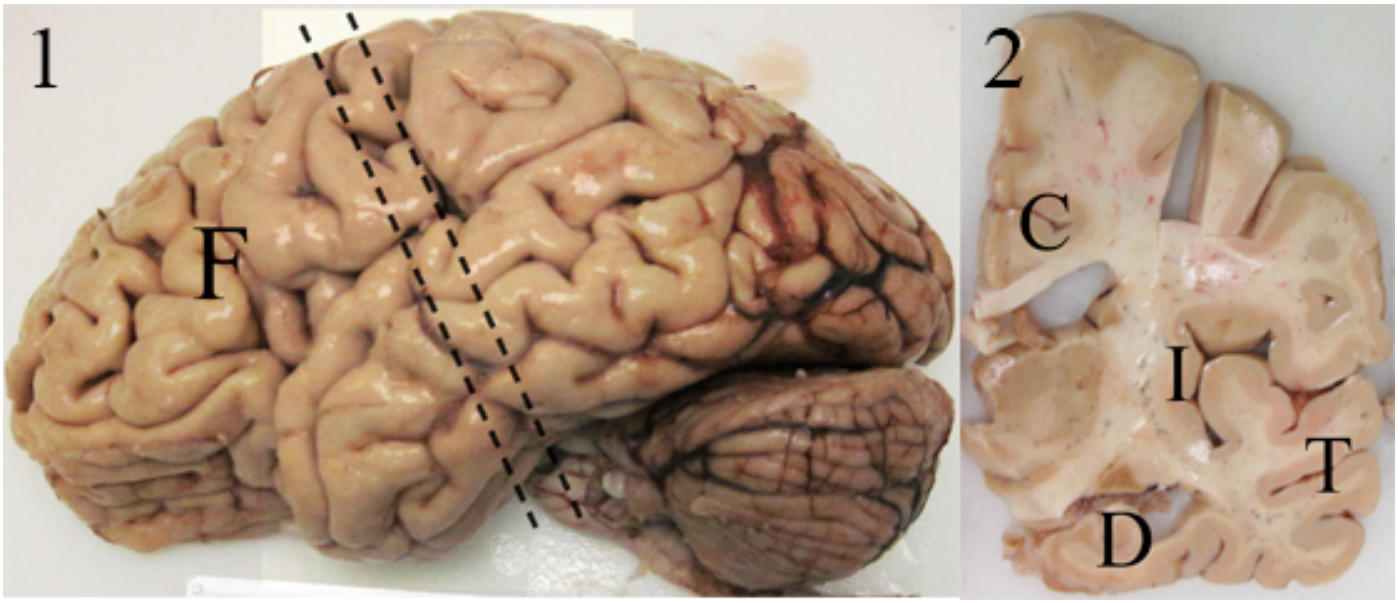

Figure 1 – Approximate locations of sectioned tissues. Figure 1.1 shows the approximate location of the frontal cortex (marked by "F") that was sectioned. The hashed lines represent the approximate depth and location of the coronal section shown on the right in Figure 2.2, which shows the following regions: dentate gyrus (D), cingulate cortex (C), insular cortex (I), and temporal cortex (T).

Regions of Interest

Five brain regions – the dentate gyrus, frontal cortex, cingulate cortex, insular cortex, and temporal cortex - were selected for investigation on the basis of their association with the dysfunction seen in bvFTD patients. The hippocampus is known to regulate emotion and stress response, and the dentate gyrus is suspected to be an important factor in this regulation (Fa et al., 2014). Damage to the frontal cortex has been shown to impair working memory and lead to psychosocial problems such as antisocial behavior, aggression, and irritability (Kimberg & Farah, 1993, Séguin, 2009). Similarly, cingulate cortex lesions lead to decreased social interactions and emotional changes (Hadland et al., 2003). The temporal cortex (specifically around the superior temporal gyrus), has been labeled as an important structure for social

cognition (Bigler et al., 2007). The approximate locations of the regions from which the sections were taken can be seen in Figure 1.

Immunohistochemistry

Immunohistochemistry was performed using 9μm paraformaldehyde-fixed, paraffin-embedded post-mortem human brain tissues from the hippocampus, frontal cortex, insular cortex, cingulate cortex, and temporal cortex. Sections were incubated with anti pTDP-43 antibody specific for pathological TDP-43 inclusions (1:2,000; Cosmo Bio) and developed using a 3,3'-diaminobenzoic acid (DAB) peroxidase substrate kit (Vector Laboratories) and counterstained with hematoxylin.

Software Analysis

Slides were gently cleaned and then scanned into a digital format (.ndpi files) via a Hamamatsu Nanozoomer 2.0-HT scanner with up to 40x magnification. The program used to view the .ndpi virtual slide files was Aperio ImageScope (v11.2.0.780). Once the slides were opened in ImageScope, a stylus or touchpad was used to draw three nonadjacent target regions for analysis in the cortical gray matter for the frontal, insular, cingulate, and temporal cortex slides as seen in Figure 2. The intent behind using three smaller annotation layers rather than one larger, continuous annotation layer was to avoid artifacts, folds and tears in the tissue, and other items that could potentially skew the software analysis that would be eventually run on the annotation layers. Due to the slow pace of the software,

averaging three separate regions in the area of interest also allowed for more efficient representation of the whole area rather than one large annotation that covered the entire gray matter of the slide.

Some slides were excluded from analysis because the pTDP-43 slides for those particular cases and regions were missing from the slide inventories. This was the case for 12 dentate gyrus slides (6 ALS cases and 6 FTLD cases), 5 frontal cortex slides (all were FTLD cases), 5 cingulate cortex slides (all were FTLD cases), 7 insular cortex slides (all were FTLD cases), and 4 temporal cortex slides (1 ALS case, 1 ALS+FTD case, and 2 FTLD cases).

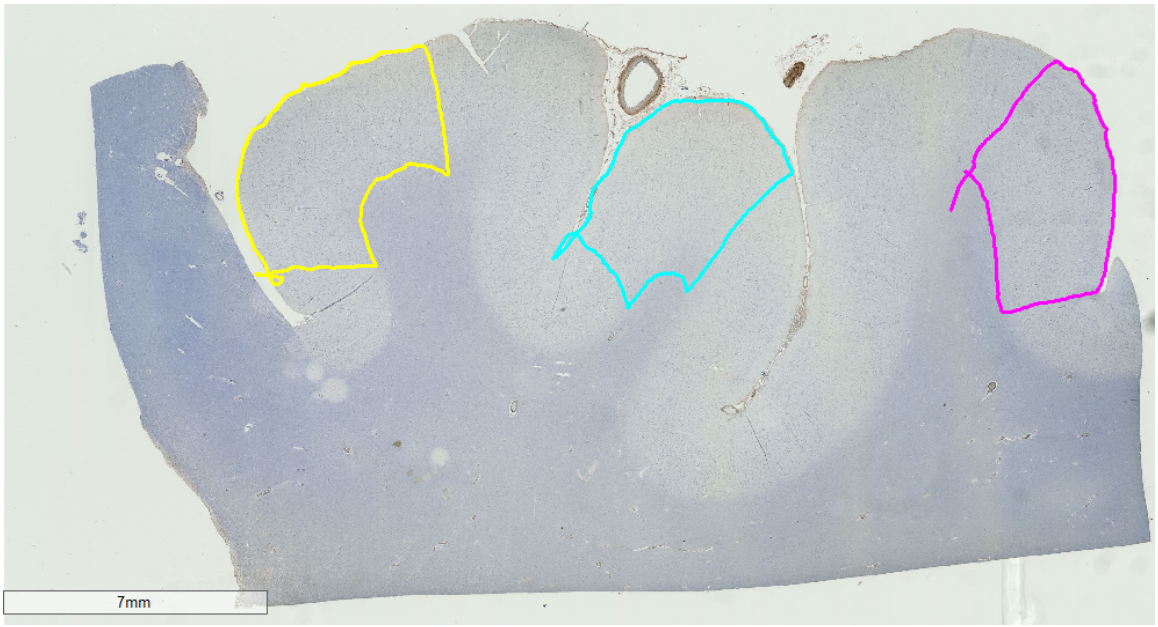

Figure 2 – Sample slide of cortical tissue that has been annotated with three separate regions for analysis.

Nuclear Counting

Two separate algorithms were run on each of the three annotation layers for each slide. The first algorithm was the Nuclear v9 "Quantitation of Nuclear Staining" algorithm. When run through a selected area, this algorithm locates nuclei, classifies the nuclei as strong (3+), moderate (2+), weak (1+), or neutral (0+) according to the intensity and colors of the pixels, and counts the number of nuclei in each category. Before any slides had been analyzed, the specifications of this algorithm were tuned by changing the labeling criteria until the algorithm consistently labeled large aggregates of pTDP-43 in or adjacent to nuclei as strong (3+) nuclei. This allowed for the calculation of the proportion of nuclei with associated aggregates by dividing the number of strong (3+) nuclei by the number of total nuclei. Because this algorithm was run on three separate regions per slide, the proportion of nuclei with associated cellular aggregates was averaged in order to find an approximate proportion for the whole section.

Nuclear v9 Parameters

View Width	10000
View Height	10000
Overlap Size	100
Image Zoom	1
Markup Compression	Same as processed image
Compression Quality	30
Classifier Neighborhood	0
Classifier	None
Class List	
Averaging Radius (um)	1.5
Curvature Threshold	0.5
Segmentation Type	Cytoplasmic Rejection
Threshold Type	Edge Threshold Method
Edge Trimming	Un-Weighted
Lower Threshold	0
Upper Threshold	230
Min Nuclear Size (um^2)	20
Max Nuclear Size (um^2)	1000000
Min Roundness	0.5
Min Compactness	0
Min Elongation	0.5
Remove Light Objects	0
Clear Area Intensity	240
Nuclear Stain (Red)	0.696858
Nuclear Stain (Green)	0.643073
Nuclear Stain (Blue)	0.317563
Positive Stain (Red)	0.244583
Positive Stain (Green)	0.509334
Positive Stain (Blue)	0.825081
Cytoplasmic Intensity Threshold	230
Weak (1+) Threshold	210
Moderate (2+) Threshold	188
Strong (3+) Threshold	210
Black Threshold	0
Use Mode	Analysis/Tuning
Mark-up Image Type	Analysis
Classifier Type	IHCNuclear
Classifier Definition File	IHCNuclearTraning

Table 2 – Specifications for the Nuclear v9 algorithm used to count nuclei in targeted regions.

The parameters for this algorithm that were found to consistently label nuclei as desired can be found in Table 2. It should be noted that this algorithm was incapable of recognizing and labeling every single nucleus, but with the above specifications, the algorithm correctly identified most nuclei and appeared to indiscriminately miss both normal nuclei and nuclei with an associated cellular pTDP-43 aggregate. A sample output of the nuclear counting can be seen in Figure 3.

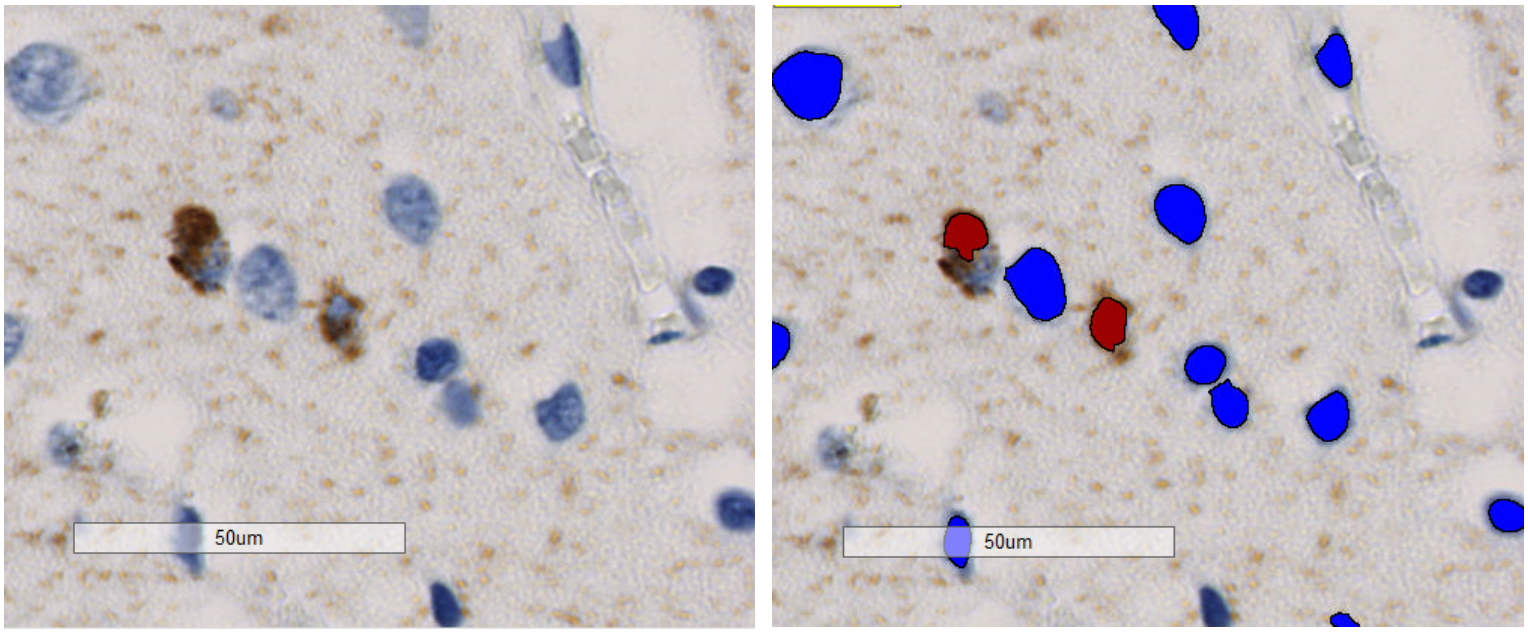

Figure 3 – Sample cortical region before (left) and after (right) analysis by the Nuclear v9 algorithm. Right: nuclei without pTDP-43 aggregates are shown in blue, and nuclei with pTDP-43 aggregates are shown in red.

Another important clarification is that this algorithm was limited in regard to the density of nuclei in the region of analysis. That is, the algorithm was unable to count multiple nuclei that overlapped. This proved to be of no consequence for cortical regions in which overlapping nuclei were uncommon. However, the dentate gyrus is much more dense than the cortical regions and therefore has many overlapping nuclei. In order to find the proportion of nuclei with associated aggregates for the

dentate gyrus, these cells were counted by hand using a stylus to mark each cell as a new annotation layer and then viewing the total number of annotation layers. After counting every cell in a segment of the dentate gyrus, the process was repeated over the same segment, this time counting the number of aggregates. The proportion of nuclei with associated aggregates was then found by dividing the number of tallied aggregates by the total number of tallied cells in the segment of the dentate gyrus.

Pixel Counting

The second algorithm that was run on each region was the Positive Pixel Count v9 algorithm. This software runs through a selected region and classifies each pixel as negative, weak, moderate, or strong according to the intensity and color of the pixel. This allows for the quantification of the number of pixels in a given area that fall within a specified range of intensity and color. In this case, it was used to find the percentage of the area of a region that was occupied by pTDP-43. This was done by tuning the pixel intensity such that pTDP-43 pixels were labeled as "strong" pixels, while all other pixels were labeled as "negative pixels." The proportion of cortical area occupied by pTDP-43 was calculated by dividing the number of strong pixels by the total number of pixels for each annotation layer and then finding the average of the proportions for the three annotation layers. Contrary to the Nuclear v9 algorithm which is robust against slight

Positive Pixel Count v9 Parameters

View Width	1000
View Height	1000
Overlap Size	0
Image Zoom	1
Markup Compression	Same as processed image
Compression Quality	30
Classifier Neighborhood	0
Classifier	None
Class List	
Hue Value	0.1
Hue Width	0.5
Color Saturation Threshold	0.04
Iwp(High)	220
Iwp(Low) = Ip(High)	220
Ip(Low) = Isp(High)	170
Isp(Low)	0
Inp(High)	-1

Table 3 – Specifications for the Positive Pixel Count v9 algorithm used to count pixels in targeted regions.

differences in staining between slides, the Positive Pixel Count v9 algorithm needed to be tuned specifically to each slide in order to produce accurate results. This was accomplished by modifying only the "Ip(Low) = Isp(High)" parameter, which dictates the threshold at which a pixel is classified as a strong pixel. The typical parameters for this algorithm can be seen in Table 3, though it should be noted that the value of "170" for "Ip(Low) = Isp(High)" is the only value that was not conserved from slide to slide. A sample output from this algorithm can be seen in Figure 4.

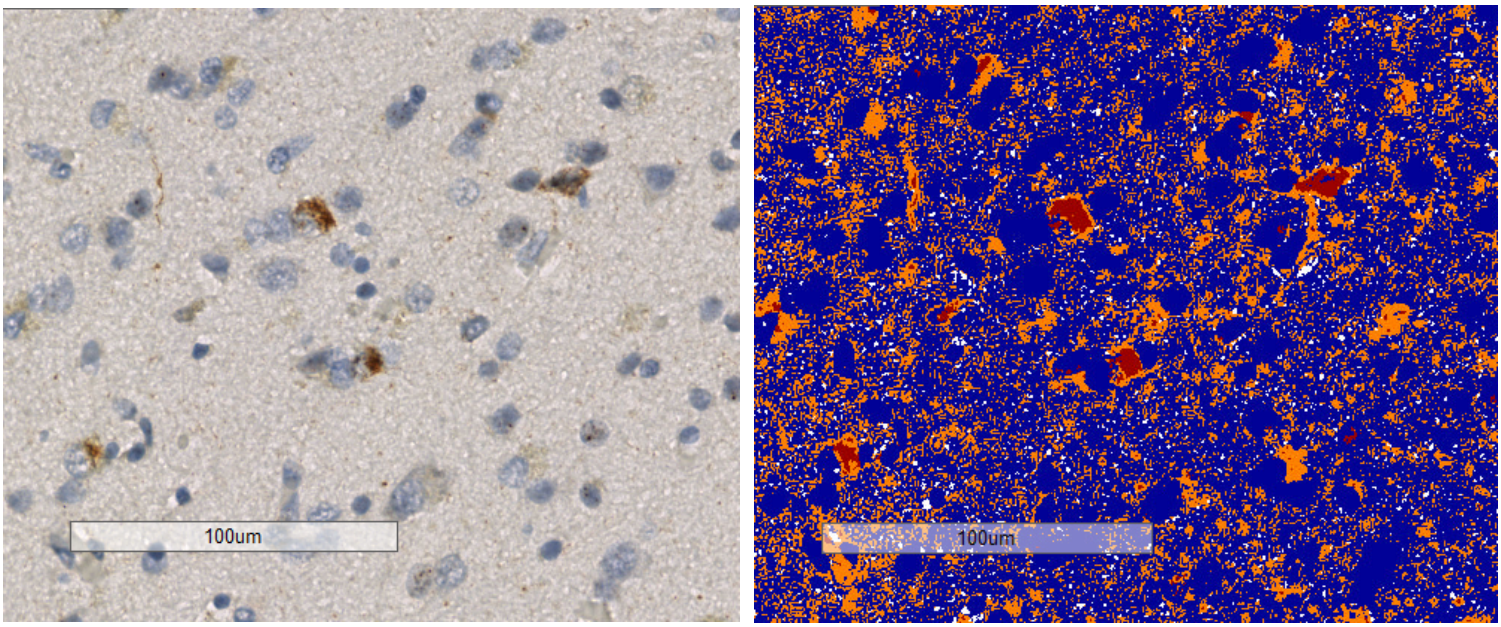

Figure 4 – Sample results from the Positive Pixel Count v9 algorithm. The image on the left shows the region (at 20x magnification) without the analysis results, and the image on the right shows the exact same area with the results. Blue and orange represent pixels that are negative for pTDP-43, whereas red represents pixels that are registered as containing pTDP-43.

As with the Nuclear v9 algorithm, the Positive Pixel Count v9 algorithm did not easily translate to the dentate gyrus. While cortex regions have clear boundaries along which the annotation layers could be drawn (the division between gray matter and white matter as one boundary and the outer edge of the cortex as another), the dentate gyrus tends to have a less-defined boundary from the rest of the hippocampus. This led to

more subjective and less accurate attempts at finding the proportion of the region occupied by pTDP-43 as there was no clear and consistent way to define the border of the dentate gyrus. Therefore the area amount of pTDP-43 in the dentate gyrus was excluded from the analysis.

Statistics

Differences between subject groups were examined by using the Kruskal-Wallis one-way nonparametric ANOVA test for each algorithm for each region, since the distributions were neither normal nor varied equally between regions. ANOVA tests that were significant at the $P<0.05$ level were followed by unpaired, non-parametric Mann-Whitney t-tests between the three subject groups for the specific region-algorithm pair. These t-tests were evaluated at the $P<0.017$ significance level given that the initial ANOVA was performed across three subject groups. Analysis was also done on the correlation between the quantity of pTDP-43 in a region (both via proportion of nuclei and proportion of area associated with the protein) and the age of onset of disease, age of death, and length of time with the disease. This was accomplished using a two-tailed, nonparametric Spearman correlation with a 95% confidence interval between each region-algorithm pair and the variable of interest (onset age, death age, and disease length). Outliers were defined as being 3 or more standard deviations from the mean and were removed from each subject group prior to analysis. All statistics were performed using Graphpad Prism 6 software.

Results

Dentate gyrus

Cellular inclusions

Analysis of the proportion of dentate gyrus cells that had cellular pTDP-43 inclusions revealed a significant difference between the ALS and ALS+FTD groups (Fig. 5). pTDP-43 inclusions were detected in 1.39 ± 0.32% of dentate gyrus cells in ALS patients (n=12), in 5.25 ± 1.24% of dentate gyrus cells in ALS+FTD patients (n=8), and in 7.08 ± 2.13% of FTLD patients (n=11). The nonparametric one-way ANOVA resulted in a Kruskal-Wallis test statistic of 7.711 with P=0.0212. Three pairwise comparisons were then performed between the three groups (ALS vs ALS+FTD, P=0.0041; ALS vs FTLD, P=0.0510; ALS+FTD vs FTLD, P=0.9374), indicating that the ALS and ALS+FTD subject groups had significantly different means. Two outliers were removed from the ALS group (6.79%, Z=4.88; 6.69%, Z=4.81), and one outlier was removed from the ALS+FTD group (40.25%, Z=10.02). However, even with the inclusion of the three outliers in the ANOVA and t-tests, there is still a statistical significance between the ALS and ALS+FTD subject groups .

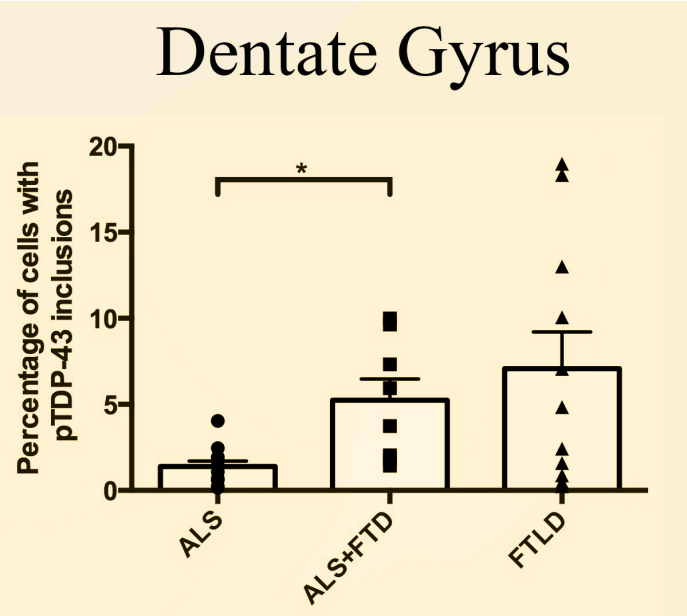

Figure 5 - Percentage of detected cells that contain pTDP-43 in the dentate gyrus. Two significant outliers (6.79%, Z=4.88; 6.69%, Z=4.81) were omitted from the ALS group, and one significant outlier (40.25%, Z=10.02) was removed from the ALS+FTD group). Data are means ± SEM (* indicates P<0.01 between the means of the two groups).

Protein quantification

As mentioned previously, the protein quantification (via area of the region occupied by pTDP-43) for the dentate gyrus was not performed due to limitations with the software analysis as a result of the nuclear density of this particular region.

Frontal cortex

Cellular inclusions

There was no significant difference in the percentage of cells with pTDP-43 inclusions in the frontal cortex between the different patient populations (Fig. 6A). Aggregates were found to be associated with 4.71 ± 1.03% of cells in ALS frontal cortices (n=20), 3.61 ± 0.68% in ALS+FTD frontal cortices (n=9), and 3.81 ± 1.19% of FTLD frontal cortices (n=11). The Kruskal-Wallis test statistic for these means was 1.511 with P=0.7732. One value from the FTLD subject group was excluded from this analysis, as the percentage of cells with inclusions for this case (15.73%, Z=3.02) was found to be an outlier using the ROUT method with Q=1%.

Protein quantification

The quantitative analysis of the area covered by pTDP-43 in the frontal cortex revealed no statistically significant difference between the ALS+FTD group and the FTLD group (Fig. 6B). The one-way ANOVA test produced a Kruskal-Wallis test statistic of 5.611 with P=0.0605, which was considered to be insignificant at the P<0.05 level. The protein occupied 1.32 ± 0.16% of the area in ALS frontal cortices (n=20), 2.10 ± 0.48% of the area in ALS+FTD frontal cortices (n=8), and in 0.94 ±

0.21% of the area in FTLD frontal cortices (n=11). One outlier from the ALS+FTD group (16.24%, Z=10.96) and one outlier from the FTLD group (5.86%, Z=9.48) were removed from this analysis.

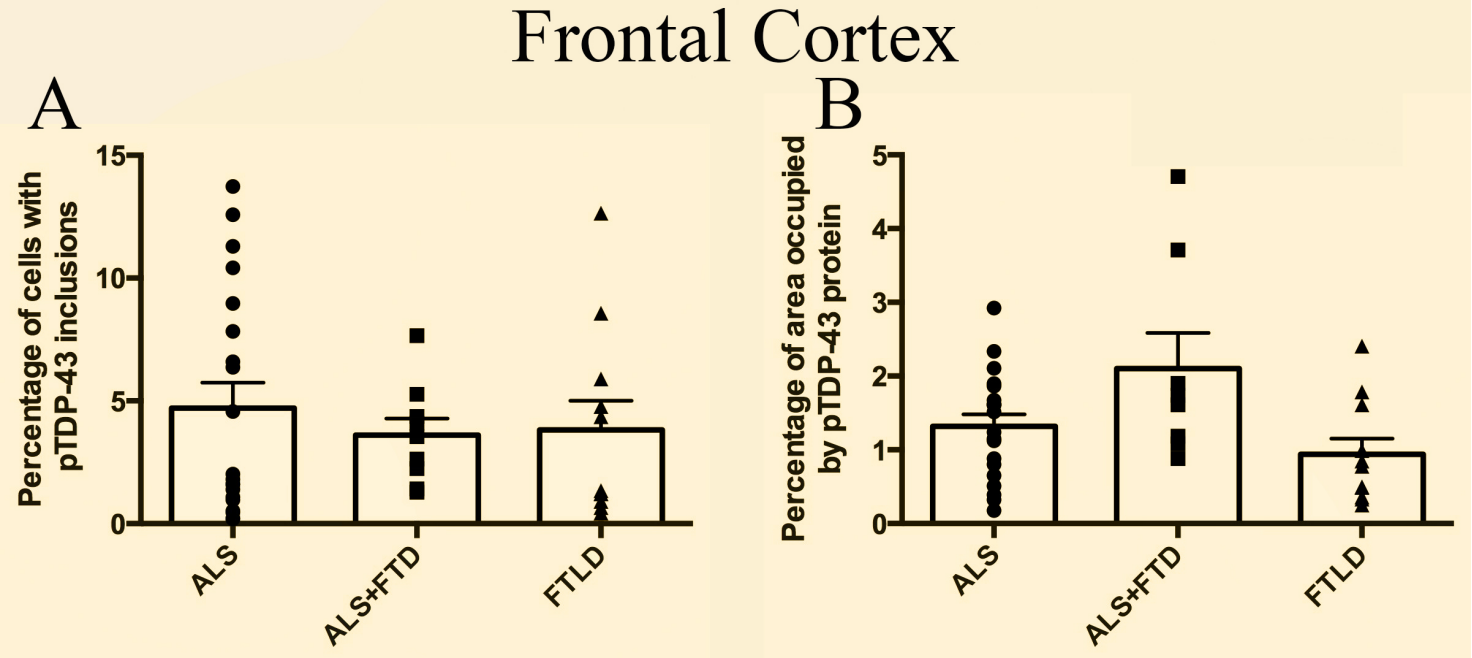

Figure 6 – (A) Percentage of detected cells that contain pTDP-43 inclusions and (B) percentage of cortical area covered with pTDP-43 protein in the frontal cortex. One significant outlier (15.73%, Z=3.02) was omitted from the FTLD group in (A). Two significant outliers (16.24%, Z=10.96; 5.86%, Z=9.48) were removed from (B) Data are means ± SEM.

Cingulate cortex

Cellular inclusions

The proportion of cells with inclusions in the cingulate cortex did not vary significantly between the subject groups (Fig. 7A). Aggregates were found in 3.65 ± 0.63% of cells in ALS cingulate cortices (n=20), 3.00 ± 0.54% of cells in ALS+FTD cingulate cortices (n=9), and 1.56 ± 0.25% of cells in FTLD cingulate cortices (n=11). The nonparametric Kruskal-Wallis one-way ANOVA revealed a significance of P=0.1914 with a test statistic of 3.307. One outlier in the FTLD group was removed from analysis with a value of 15.65% and Z=16.81.

Protein quantification

There were no differences among the mean percentages of area occupied by pTDP-43 in the cingulate cortex for the three subject groups (Fig. 7B). The pTDP-43 protein occupied 1.33 ± 0.19% of the area in ALS cingulate cortices (n=20), 1.53 ± 0.35% of the area in ALS+FTD cingulate cortices (n=9), and 2.90 ± 0.71% of the area in FTLD cingulate cortices (n=12). The Kruskal-Wallis test statistic for this analysis was 2.696 with *P*=0.2598.

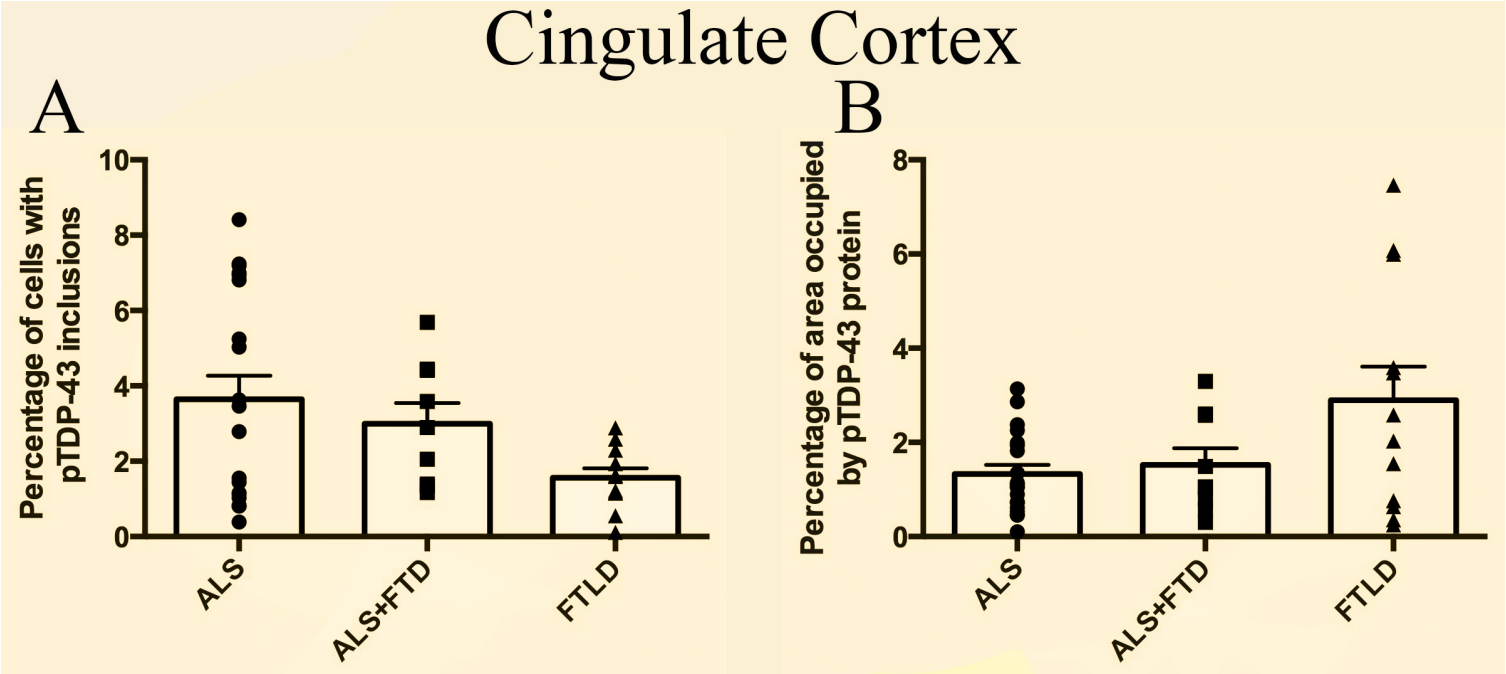

Figure 7 – (A) Percentage of detected cells that contain pTDP-43 inclusions and (B) percentage of cortical area covered with pTDP-43 protein in the cingulate cortex. One significant outlier (15.65%, Z=16.81) was omitted from the FTLD group in (A). Data are means ± SEM.

Insular cortex

Cellular inclusions

The proportion of cells with pTDP-43 inclusions in the insular cortex did not vary significantly between patients groups (Fig. 8A). The initial one-way ANOVA produced *P*-value of 0.1174 with a test statistic of 4.285. Inclusions were detected in 3.70 ± 0.74% of cells of ALS insular cortices (n=18), 5.17 ± 1.14% of cells in ALS+FTD insular cortices (n=9), and in 2.41 ± 0.67% of FTLD insular cortices (n=10). Two outliers from the ALS-only subject group were excluded from the analysis as the values (13.43% and 13.95%) were more than 3 standard deviations from the mean of the subject group (Z=3.11 and Z=3.47, respectively).

Protein quantification

Analysis of the amount of protein in the insular cortex showed that there were no differences between the mean percentages of area occupied by pTDP-43 in the insular cortices of ALS, ALS+FTD, and FTD patients (Fig. 8B). The one-way ANOVA had a Kruskal-Wallis test statistic of 0.6736 and a *P*-value of 0.7140. The phosphorylated protein accounted for 1.42 ± 0.40% of the area in ALS insular cortices (n=20), 1.31 ± 0.36% of the area in ALS+FTD insular cortices (n=9), and 1.13 ± 0.30% of the area in FTLD insular cortices (n=8). One outlier was identified within the insular cortex FTLD group for the area analysis with a percentage value of 9.25% and Z=9.66.

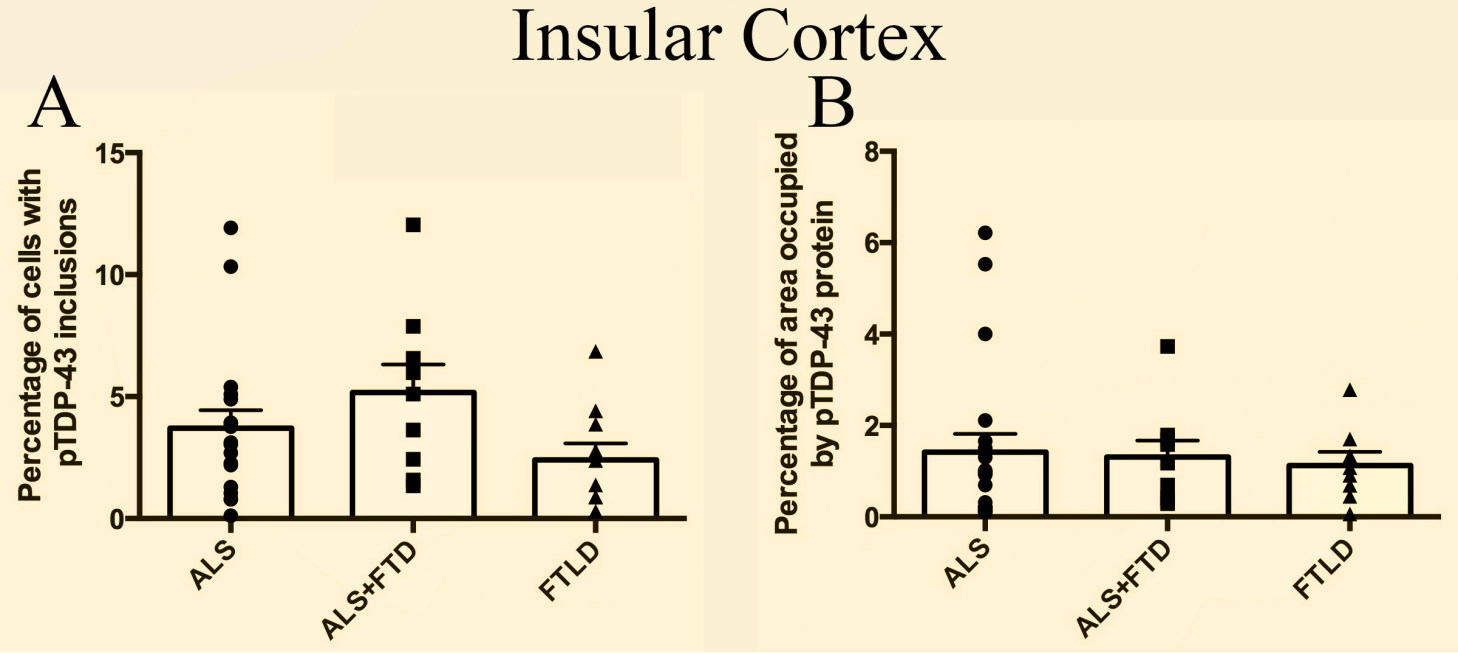

Figure 8 – (A) Percentage of detected cells that contain pTDP-43 inclusions and (B) percentage of cortical area covered with pTDP-43 protein in the insular cortex. Two significant outliers (13.43%, Z=3.11; 13.95%, Z=3.47) were removed from the ALS group in (A). One significant outlier (9.25%, Z=9.66) was removed from the FTLD group in (B). Data are means ± SEM.

Temporal cortex

Cellular inclusions

The proportionate number of cells with protein aggregates in the temporal cortex did not vary significantly across the subject groups (Fig. 9A). TDP-43 inclusions were associated with 0.71 ± 0.16% of cells in ALS temporal cortices (n=16), 0.78 ± 0.16% of cells in ALS+FTD temporal cortices (n=7), and 1.68 ± 0.44% of cells in FTLD temporal cortices (n=14). The nonparametric one-way ANOVA resulted in a test statistic of 2.599 with P=0.2727. Three outliers were removed from analysis of the ALS subjects, as their values of 3.17%, 5.72%, and 3.05% corresponded to Z-scores of 3.84, 7.81, and 3.65, respectively. One outlier was removed from analysis in each of the ALS+FTD (3.53%, Z=6.37) and FTLD (8.90%, Z=4.34) groups,

Protein quantification

The quantification of the protein in the temporal cortex via percentage of area analysis revealed no statistically significant difference among the three patient populations (Fig. 9B). Phosphorylated TDP-43 aggregates accounted for 1.36 ± 0.24% of the area in ALS temporal cortices (n=19), 2.44 ± 0.67% of the area in ALS+FTD temporal cortices (n=8), and 0.95 ± 0.25% of the area in FTLD temporal cortices (n=14). The Kruskal-Wallis test for these three subject groups resulted in a test statistic of 5.769 with *P*=0.0559. One outlier was removed from analysis of the FTLD group, as the percentage of area occupied by pTDP-43 in this case was 11.95%, which was found to be 11.91 standard deviations from the mean.

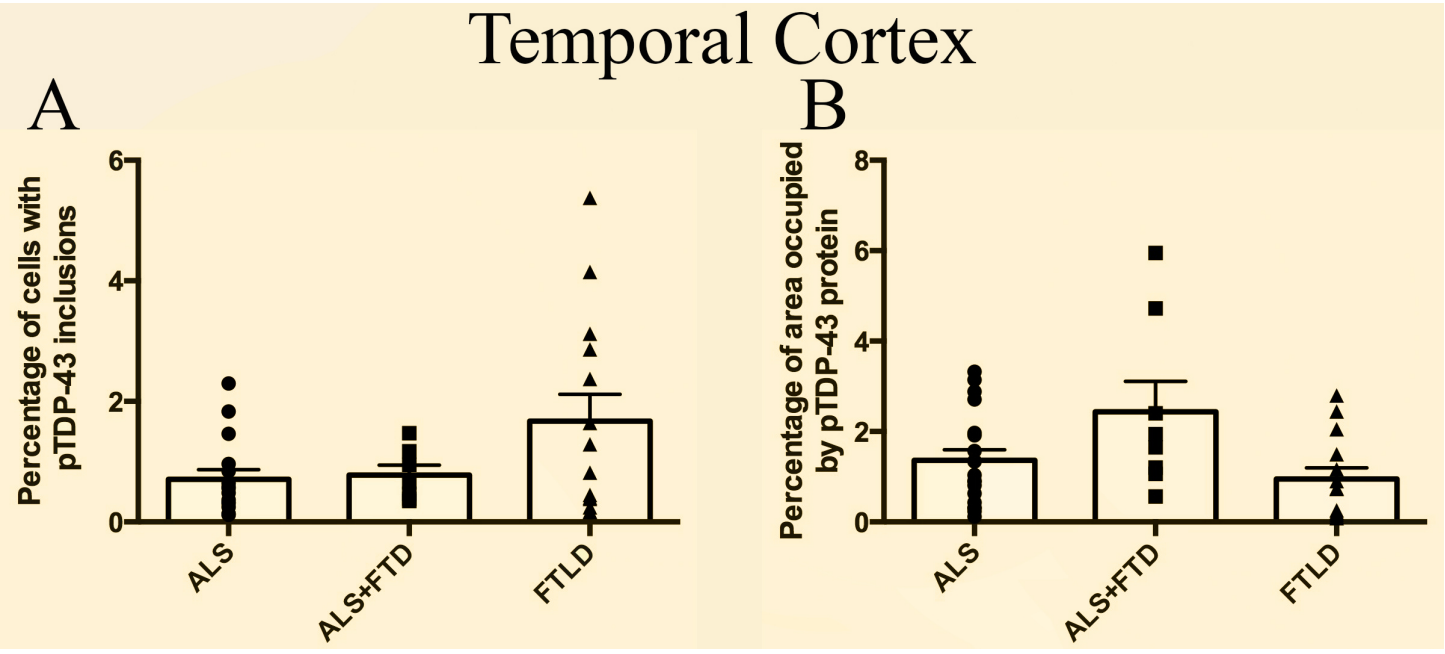

Figure 9 – (A) Percentage of detected cells that contain pTDP-43 inclusions and (B) percentage of cortical area covered with pTDP-43 protein in the temporal cortex. One significant outlier (3.53%, Z=6.37) from the ALS+FTD group and another (8.90%, Z=4.34) from the FTLD group were removed from (A). A third significant outlier (11.95%, Z=11.91) was removed from the FTLD group in B. Data are means ± SEM.

Discussion

We examined the quantity and distribution of pTDP-43 inclusions to determine if the presence of this protein in the brain is a marker for dementia in ALS patients. By using two methods, aggregate counting and pixel counting, we accounted for both the number of pTDP-43 inclusions and the amount of the protein.

Quantitative analysis of the dentate gyrus was limited to aggregate counting, yet this method produced the only statistically significant result: non-demented ALS patients had a lower proportion of pTDP-43 aggregates per nucleus than did demented ALS patients, but neither group was different from the FTLD patients in this regard (Fig. 2). In no other region of the brain were we able to find a difference in the number of pTDP-43 inclusions or amount of pTDP-43 protein among the three subject groups. At the surface, this would seem to indicate that elevated levels of pTDP-43 in the dentate gyrus are a marker for dementia in ALS patients. However, this statement is contradicted by patients such as **ALS+FTD Case 5** and **ALS+FTD Case 9** (Table S2), who were demented ALS patients that exhibited levels of pTDP-43 near or below the mean pTDP-43 level for non-demented ALS cases. Thus, if pTDP-43 in the dentate is a marker of FTD, how can cognitively normal ALS patients have a higher pTDP-43 burden in the dentate gyrus than demented ALS patients? Given that our results indicate that demented patients have a higher *average* pTDP-43 burden in the dentate gyrus than non-demented ALS patients, it is a possibility that this protein is only one factor that determines the cognitive fate of

ALS patients. In keeping with the literature, the pTDP-43 burden may be a marker for dementia alongside genetic predisposition or susceptibility. More specifically, one could imagine that ALS patients with a relatively high dentate pTDP-43 burden may be susceptible to dementia if they also have a mutated C9ORF72 gene – akin to a gating or cooperative effect of both factors. This could explain why only a subset of people with the C9ORF72 expansion develops dementia, as well as why ALS patients may be cognitively normal despite relatively high pTDP-43 pathology in their dentate gyrus.

Another perplexing aspect of the dentate gyrus results is that the pTDP-43 burden in this region appears to be independent of the pathological burden in other regions. **ALS Case 3**, **ALS Case 6,** and **ALS Case 7** all have low percentages of cells with pTDP-43 inclusions in the dentate gyrus, yet all three have relatively high pTDP-43 levels in 2-3 other regions of the brain (Fig. S1). Furthermore, **ALS+FTD Case 2** and **ALS+FTD Case 8** have much higher levels of pTDP-43 in the dentate gyrus than in all of the other four regions (Fig. S3). Thus, there is no obvious relationship between the pTDP-43 pathology of the dentate gyrus and that of the other regions.

One of the outliers from the dentate gyrus ALS+FTD group, **ALS+FTD Case 2**, had a proportion of cells with pTDP-43 aggregates equal to over 40%, which is approximately 10 standard deviations higher than the mean for this group (5.25%). In addition to ALS, this patient received a secondary neuropathological diagnosis of FTLD-TDP and a third neuropathological diagnosis of Alzheimer's disease, which

may account for the high volume of pTDP-43 in their dentate gyrus. Two other cases, **ALS+FTD Case 4** and **ALS+FTD Case 7**, also had the same three neuropathological diagnoses, yet both of their pTDP-43 levels (7.33% and 3.75%, respectively) in this region were within two standard deviations of the mean. Therefore **ALS+FTD Case 2**'s particularly high pTDP-43 burden is still perplexing from a pathological perspective, though it may be true that this patient's dementia severity was significantly worse than the other cases, which would account for the unusual pathology.

The lack of differences among subject groups for the quantity and distribution of pTDP-43 in the frontal, cingulate, insular, and temporal cortices is puzzling in itself. That is, how can brain regions, especially those that are closely associated with mental processes such as social cognition and emotion regulation, be similarly riddled with pTDP-43 in demented and non-demented patients? Research has shown that lesions to the cingulate cortex result in emotional changes and decreased social interactions, two defining characteristics of bvFTD, so it would logically follow that a high proportion of cells with associated pTDP-43 aggregates in this area would result in some form of these behavioral changes (Hadland et al., 2003). We see that this is not the case as evidenced by the results in Figure 4 as some ALS patients have elevated cingulate pTDP-43 levels yet remain cognitively normal. Moreover we see FTLD patients with very little pTDP-43 pathology in the cingulate. This is true across all four of the non-dentate regions. These results would seem to point to the conclusion that the levels of pTDP-43 pathology seen in this

study are not sufficient to impair function in a cortical region or serve as a marker for impaired function in a cortical region.

The frontal cortex data for the ALS and FTLD groups in Figure 6A resemble a bimodal distribution. There is a realistic chance that this is due entirely to the sampling distribution, but it is worth noting that the ALS group in particular shows an interesting phenomenon. We see that about half of the values are relatively small or close to zero, while the other half of the points span a larger range yet have surprisingly high values. This again brings up the notion that high pTDP-43 burdens do not necessarily imply clinical dementia. Given that studies have estimated that the C9orf72 expansion accounts for up to 50% of genetic ALS-FTD cases (Verma 2014), we return to the possibility that both pTDP-43 pathology and C9orf72 mutation are required for the presentation of clinical symptoms. This would explain the bimodal distribution in the ALS column – approximately half of the patients are predisposed to heavier pTDP-43 pathology, but they may lack clinical symptoms because they lack the C9orf72 mutation. Unfortunately, this study does not contain the genetic data for the patients, so this conclusion cannot easily be supported or weakened further with our data. There is, however, a second possible explanation for the bimodal distribution based on the literature. Brettschneider et al. (2013) proposed that pTDP-43 pathology in ALS disseminates sequentially in an identifiable pattern that consists of 4 stages. The first two stages involve the presence of pTDP-43 pathology in the motor cortex, brainstem, and cerebellum, but the proposed third stage is characterized by pTDP-43 pathology having spread to

the prefrontal cortex. Using this model, we could claim that the ~50% of our ALS cases who show little-to-no pTDP-43 in the frontal cortex had yet to reach this stage 3 at the time of death, whereas the remaining ALS cases show increased levels of protein aggregates in the frontal cortex because they had advanced to stage 3 or further. The same scheme (Brettschneider et al., 2013) proposed that the pTDP-43 pathology then spreads to the temporal cortex. This is also supported by our results, as we can see another potentially bimodal distribution in the ALS group for the temporal cortex (Fig. 9A & 9B). Our **ALS Case 2** and **ALS Case 15** (Table S1) are two of the three cases that lie in what would be the upper portion of the bimodal distribution for the temporal cortex, and these two cases not only lie in the upper half of the bimodal distribution for the frontal cortex as well, but they also are both more than 1.2 standard deviations above the mean for the percentage of cells with associated pTDP-43 inclusions for the frontal cortex. We could take this to support the hypothesis that pTDP-43 pathology spreads to the temporal cortex after reaching (and building up within) the frontal cortex. However, we cannot ignore the third data point that lies in the upper portion of the potential bimodal distribution for the temporal cortex, as this case has a heavier pTDP-43 burden in the temporal cortex along with a lighter burden in the frontal cortex, which contradicts the Brettschneider dissemination hypothesis. One final point is that there is a significant difference between the proportion of cells with pTDP-43 inclusions in ALS frontal cortices and the proportion of cells with inclusions in ALS temporal cortices. A nonparametric t-test produced a *P*-value of 0.0057, implying that there is a higher percentage of cells with inclusions in the frontal cortex than in the temporal cortex

in ALS patients without dementia. This turned out to be true for ALS+FTD patients as well (P=0.0025). These results do support the Brettschneider staging proposition, as we can imagine that a higher pTDP-43 burden in the frontal cortex than the temporal cortex is consistent with the idea that this pathology builds up in the frontal cortex before spreading to the temporal cortex.

The data in this study also shed light on the idea that dementia associated with ALS is a different disease than dementia in the absence of ALS. While our focus is narrowed to the pathology of one protein in five regions, our results show that there are no significant differences between ALS+FTD and FTLD in any of the five regions studied. Thus, if ALS-associated dementia is distinct from FTLD-associated dementia, it may not be due to the pTDP-43 pathology in any of the regions that were studied in this paper.

Four of the ALS cases – **ALS Case 6, ALS Case 12, ALS Case 17,** and **ALS Case 19** – received secondary neuropathological diagnoses of FTLD. This is of significance since these are patients who had enough pTDP-43 in their brains to meet post-mortem criteria for FTLD, but were not demented. This immediately rules out the already fragile proposition that the presence of pTDP-43 inclusions implies a history of clinical dementia, and also emphasizes the more subtle and important point that the pTDP-43 neuropathology as a stand alone entity does not tell us about the presence or absence of clinical dementia. While we may see that patients with dementia have a higher average percentage of cells with pTDP-43 inclusions, the

data suggests that we would be unable to deduce that an ALS patient was demented simply because they had a high level of pTDP-43 in the dentate gyrus. Rather, our data implies that a subject may be *more likely* to have been demented if their dentate had a high level of pTDP-43. If there is a threshold pTDP-43 level in the dentate above which guarantees a correct post-mortem conclusion that patients had clinical dementia, our sample size was too small to detect it. Given that all ALS and ALS+FTD cases had at least some pTDP-43 pathology, we can reasonably conclude that pTDP-43 may be necessary but not sufficient for ALS patients to present with dementia.

Limitations and Future Directions

The small sample sizes – especially for the ALS+FTD group – were one of the primary limitations of this study. The limited number of patients was due largely in part to the number of patients with these diseases who were autopsied at Emory, and the sample sizes were further weakened due to the fact that brain tissue slides for certain regions in certain patients were unavailable. Another limitation in the study is the accuracy of the computer software. Though the algorithms were tuned to be as accurate as possible, the software was prone to making minor errors such that it did not detect the occasional nucleus or would mislabel an artifact as an aggregate.

This study laid the groundwork for potential future studies. In addition to repeating the investigation with larger sample sizes, the inclusion of genetic data such as the

presence or absence of a C9orf72 mutation would lend further insight into the factors that determine if pTDP-43 is a marker for dementia in ALS patients. A second follow-up study could be done that uses the same population groups, but targets the brain regions described in the Brettschneider et al. (2013) paper in order to further elucidate information about the staging system, but also to compare the possible sequential dissemination between demented and non-demented ALS patients in these specific regions. A third interesting direction would be to perform a similar experiment that focuses more on ALS+FTD and FTLD as two patient groups and compares the pTDP-43 burden with the patient's final performance on the Frontotemporal Dementia Rating Scale (FRS) in order to assess whether the magnitude of the pTDP-43 pathology is correlated with the severity of the cognitive symptoms.

Conclusion

The results reported in this study imply that there is a difference in the pTDP-43 pathology of cognitively normal ALS patients and demented ALS patients, and that difference is localized to the dentate gyrus. While these findings do provide some sort of answer our initial question in that there is indeed a correlation between the pTDP-43 distribution (in the dentate gyrus, we learned) and the presence of dementia in ALS patients, we find that the results raise more questions such as: 1.) Why do high pTDP-43 burdens elsewhere not provide reliable insight into the clinical data? and 2.) How can patients with high burdens of pTDP-43 remain cognitively intact while patients with low burdens of pTDP-43 present with

cognitive impairment? We also report that the data support the staging hypothesis proposed in Brettschneider et al. (2013), though further investigation remains necessary due to a small sample size. Though somewhat inconclusive, our data provides the framework for several interesting follow-up studies that may shed valuable light on the correlation between pTDP-43 pathology and clinical dementia in ALS patients.

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Supplementary Figures & Tables

	% Cells with inclusions					% Area occupied by pTDP-43							
Case #	Dentate Gyrus	Frontal	Cingulate	Insular	Temporal	Dentate Gyrus	Frontal	Cingulate	Insular	Temporal	Onset	Death	Duration
ALS Case 1	N/A	0.97	5.03	3.94	0.48	N/A	1.52	0.72	0.19	1.34	73	76	3
ALS Case 2	6.76	10.42	7.24	3.77	1.46	N/A	1.67	1.93	6.22	1.92	68	70	2
ALS Case 3	0.15	12.58	8.41	11.92	3.17	N/A	1.86	0.74	0.69	0.81	47	49	2
ALS Case 4	N/A	7.84	0.81	4.90	0.64	N/A	0.88	1.04	1.02	0.31	40	46	6
ALS Case 5	2.45	0.52	1.44	1.05	0.12	N/A	0.51	0.90	0.12	0.11	54	56	2
ALS Case 6	1.08	13.73	7.19	13.43	0.96	N/A	1.90	1.35	1.66	0.24	75	78	3
ALS Case 7	1.47	6.59	6.95	13.95	5.72	N/A	0.80	2.38	5.53	3.14	66	69	3
ALS Case 8	4.04	1.60	5.25	3.12	0.31	N/A	2.34	1.82	0.92	0.91	45	48	3
ALS Case 9	0.61	0.20	1.03	0.79	N/A	N/A	0.65	2.26	0.13	N/A	52	54	2
ALS Case 10	N/A	6.36	2.79	0.12	0.13	N/A	2.92	1.07	0.96	3.32	60	62	2
ALS Case 11	0.69	8.97	0.82	2.69	0.25	N/A	1.15	1.11	0.32	0.89	49	55	6
ALS Case 12	1.93	1.09	1.13	5.10	0.84	N/A	1.24	0.45	2.11	1.35	60	64	4
ALS Case 13	N/A	2.02	1.56	2.27	0.56	N/A	1.61	0.46	0.99	0.63	66	68	2
ALS Case 14	1.40	1.61	0.82	2.20	0.33	N/A	1.61	0.61	0.23	0.43	50	57	7
ALS Case 15	N/A	11.29	6.81	3.91	2.30	N/A	1.62	1.99	0.23	1.97	72	74	2
ALS Case 16	N/A	0.22	0.39	1.29	0.14	N/A	0.32	0.53	0.16	0.32	51	61	10
ALS Case 17	1.92	1.40	7.00	0.80	0.58	N/A	2.11	3.14	1.30	1.57	N/A	75	N/A
ALS Case 18	0.24	0.46	3.47	3.04	3.05	N/A	1.12	0.10	0.09	2.71	51	55	4
ALS Case 19	6.69	1.80	1.15	10.34	1.83	N/A	0.18	1.15	4.01	2.89	57	62	5
ALS Case 20	0.64	4.57	3.63	5.40	0.37	N/A	0.39	2.86	1.51	1.04	70	75	5

Table S1 - Percentage of cells with pTDP-43 inclusions and percentage of area occupied by pTDP-43 in each region for all studied cognitively normal ALS cases. The age at disease onset, age at death, and duration of the disease (all in years) can be seen at the right of the table. Data points labeled "N/A" indicate instances where analysis was not performed due to unobtainable slides or, in the case of the percentage of area occupied by pTDP-43 in the dentate gyrus, for all cases, due to an inability to obtain accurate, objective data.

	% Cells with inclusions					% Area occupied by pTDP-43							
Case #	Dentate Gyrus	Frontal	Cingulate	Insular	Temporal	Dentate Gyrus	Frontal	Cingulate	Insular	Temporal	Onset	Death	Duration
ALS+FTD Case 1	9.65	4.36	1.39	12.04	N/A	N/A	3.71	0.31	1.70	N/A	58	59	1
ALS+FTD Case 2	40.25	2.63	1.35	7.88	1.17	N/A	1.90	1.03	3.73	5.95	62	68	6
ALS+FTD Case 3	2.05	5.27	4.42	6.57	1.47	N/A	1.61	2.58	1.59	2.40	54	61	7
ALS+FTD Case 4	7.33	7.66	3.59	3.63	0.95	N/A	16.24	1.49	0.69	1.65	70	73	3
ALS+FTD Case 5	1.44	1.28	2.06	1.34	0.47	N/A	1.74	3.30	0.41	1.94	63	65	2
ALS+FTD Case 6	5.94	4.09	4.44	5.98	0.35	N/A	4.71	0.58	1.18	1.07	57	61	4
ALS+FTD Case 7	3.75	2.24	2.90	2.43	0.66	N/A	1.18	2.60	0.29	0.56	52	57	5
ALS+FTD Case 8	9.99	3.55	5.68	5.10	3.53	N/A	0.88	0.79	0.40	4.72	54	57	3
ALS+FTD Case 9	1.82	1.41	1.17	1.58	0.38	N/A	1.08	1.06	1.79	1.21	76	83	7

Table S2 - Percentage of cells with pTDP-43 inclusions and percentage of area occupied by pTDP-43 in each region for all studied demented ALS cases.

	% Cells with inclusions					% Area occupied by pTDP-43							
Case #	Dentate Gyrus	Frontal	Cingulate	Insular	Temporal	Dentate Gyrus	Frontal	Cingulate	Insular	Temporal	Onset	Death	Duration
FTLD Case 1	2.42	1.34	N/A	N/A	N/A	N/A	0.78	N/A	N/A	N/A	N/A	N/A	N/A
FTLD Case 2	N/A	0.90	N/A	N/A	N/A	N/A	0.85	N/A	N/A	N/A	58	63	5
FTLD Case 3	N/A	N/A	1.63	3.87	2.86	N/A	N/A	6.00	1.33	1.50	63	79	16
FTLD Case 4	10.05	N/A	2.59	N/A	8.90	N/A	N/A	7.46	N/A	11.95	56	59	3
FTLD Case 5	N/A	N/A	1.59	N/A	3.12	N/A	N/A	3.48	N/A	2.05	58	63	5
FTLD Case 6	N/A	4.76	0.55	2.79	0.40	N/A	5.86	0.25	36.80	0.11	56	64	8
FTLD Case 7	7.06	5.89	1.22	4.42	0.38	N/A	0.36	0.36	9.37	0.16	56	61	5
FTLD Case 8	18.33	N/A	N/A	N/A	2.37	N/A	N/A	N/A	N/A	1.16	56	64	8
FTLD Case 9	N/A	1.21	1.94	0.88	5.38	N/A	0.50	0.64	0.69	0.74	62	71	9
FTLD Case 10	0.88	4.36	1.15	N/A	0.17	N/A	0.33	0.76	N/A	0.08	86	>90	>14
FTLD Case 11	N/A	0.43	0.12	0.30	0.24	N/A	0.25	1.55	2.78	0.21	57	66	9
FTLD Case 12	0.47	N/A	N/A	N/A	0.16	N/A	N/A	N/A	N/A	0.11	67	71	4
FTLD Case 13	18.97	12.64	N/A	0.30	0.82	N/A	0.49	N/A	2.78	0.73	<58	60	>12
FTLD Case 14	13.00	15.73	1.18	0.90	0.45	N/A	1.79	2.03	24.73	0.26	66	67	1
FTLD Case 15	0.24	0.66	2.29	2.38	1.64	N/A	0.99	3.59	8.52	2.80	58	62	4
FTLD Case 16	1.60	1.21	2.89	1.38	1.29	N/A	1.61	2.58	13.62	0.90	57	63	6
FTLD Case 17	4.84	8.57	15.65	6.86	4.15	N/A	2.41	6.08	9.25	2.44	55	61	6

Table S3 - Percentage of cells with pTDP-43 inclusions and percentage of area occupied by pTDP-43 in each region for all studied FTLD cases.

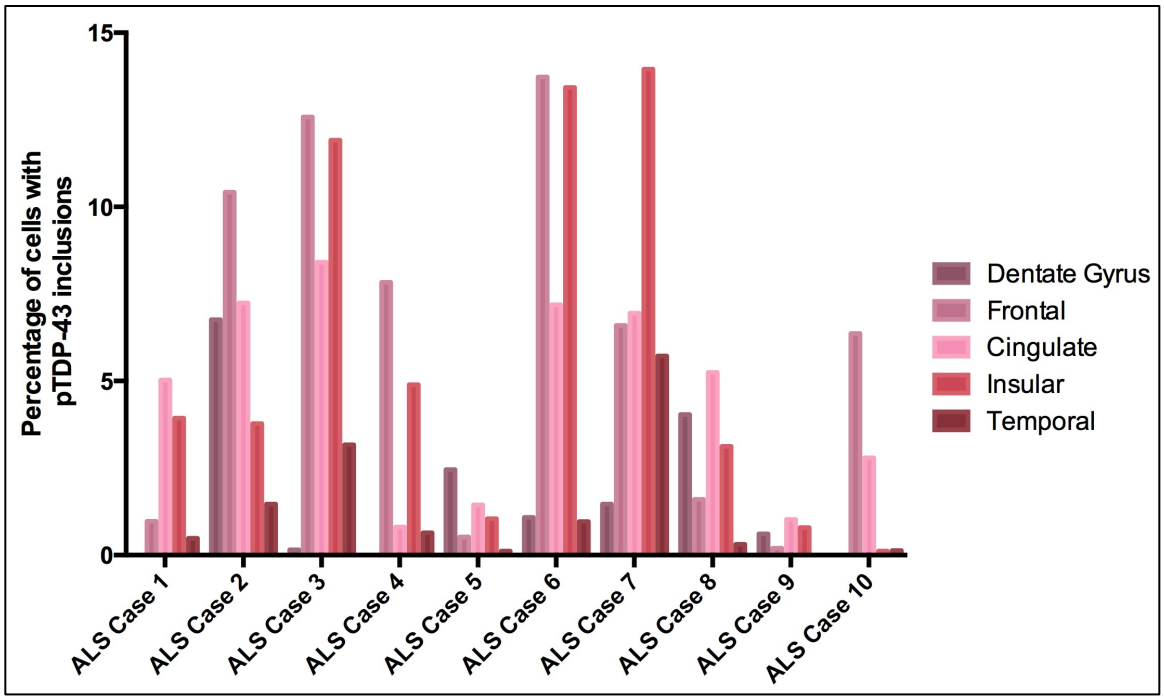

Figure S1 - Qualitative depiction of the distribution pattern of cellular pTDP-43 inclusions by case for ALS Cases 1-10

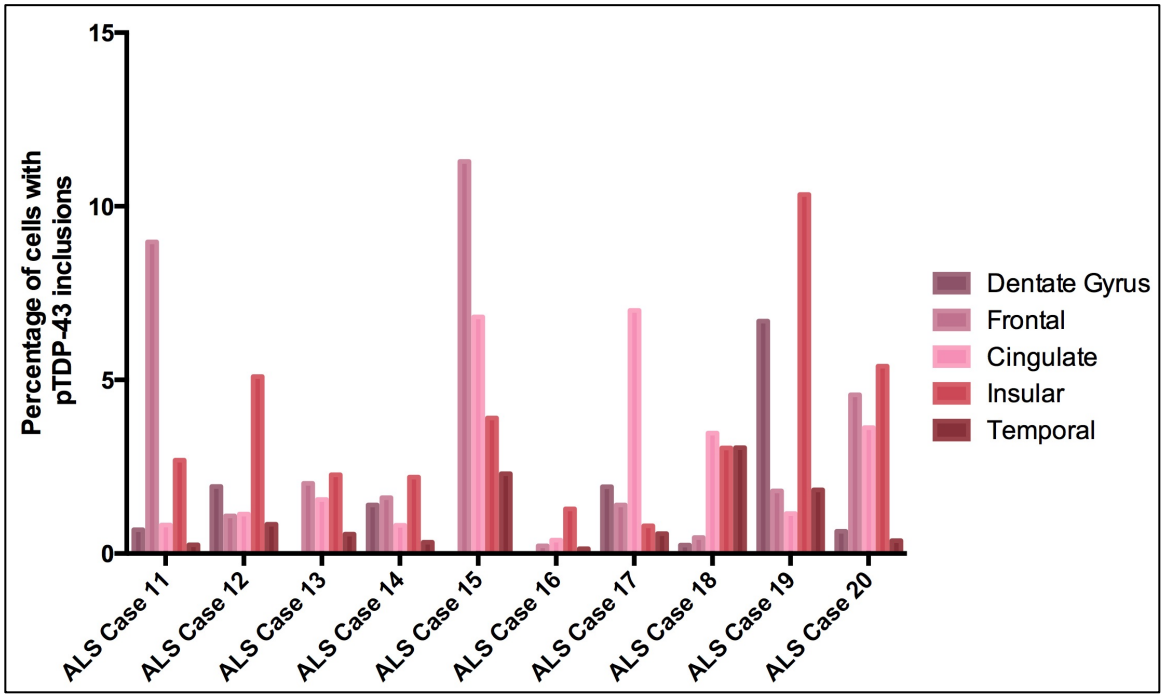

Figure S2 - Qualitative depiction of the distribution pattern of cellular pTDP-43 inclusions by case for ALS Cases 11-20

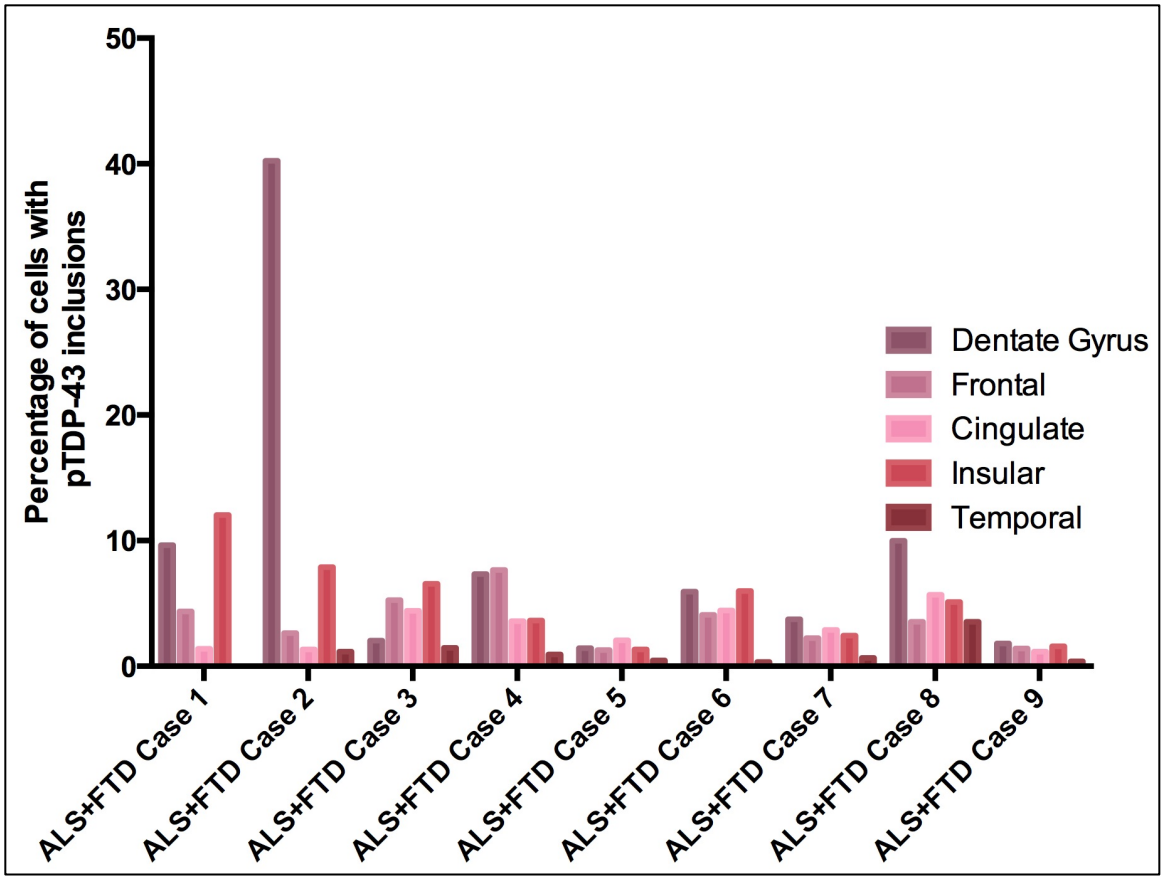

Figure S3 - Qualitative depiction of the distribution pattern of cellular pTDP-43 inclusions by case for all ALS+FTD cases

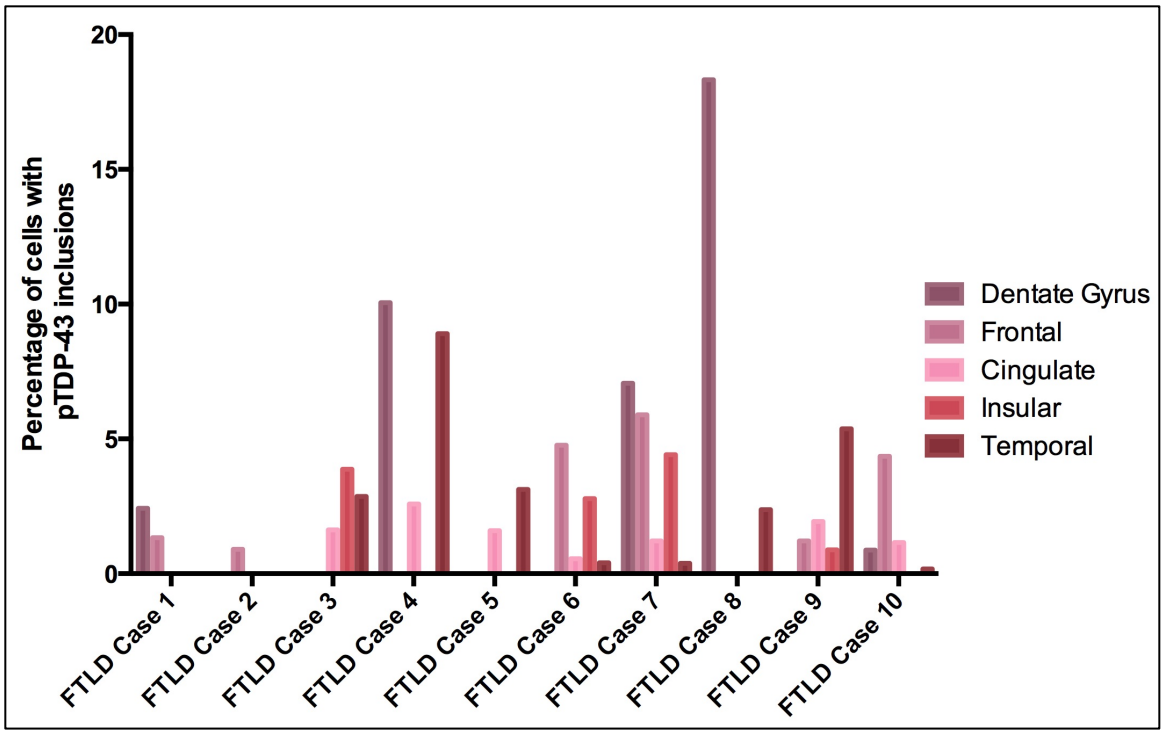

Figure S4 - Qualitative depiction of the distribution pattern of cellular pTDP-43 inclusions by case for FTLD Cases 1-10

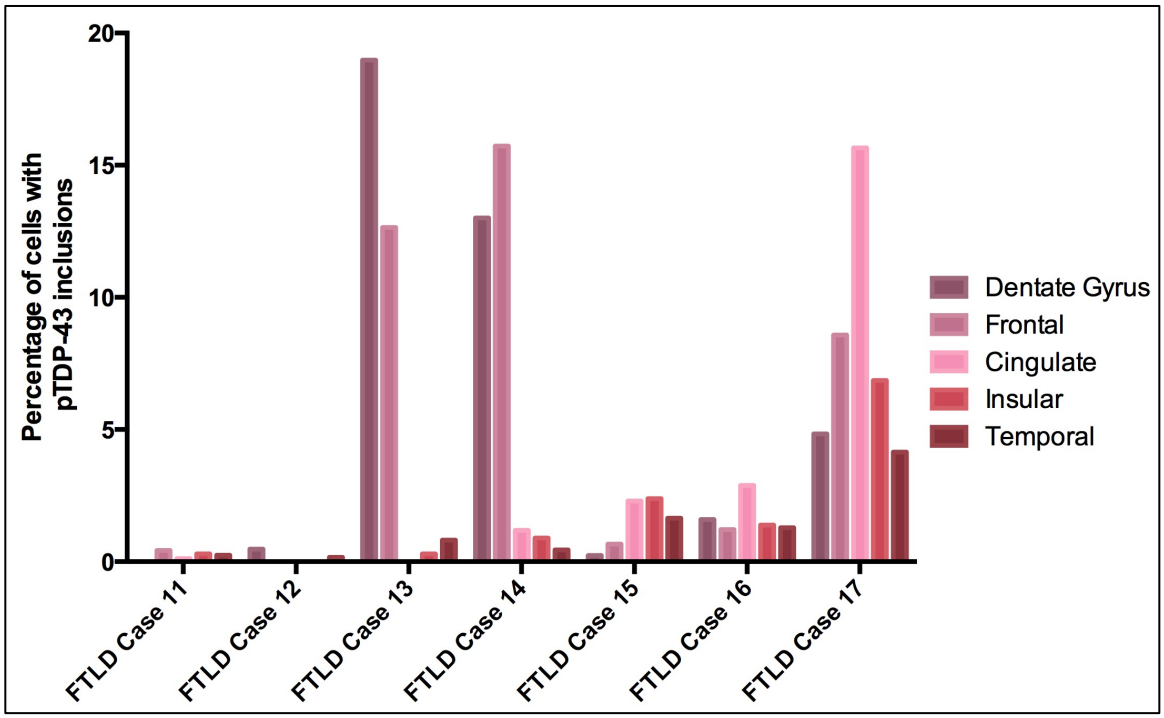

Figure S5 - Qualitative depiction of the distribution pattern of cellular pTDP-43 inclusions by case for FTLD Cases 11-17

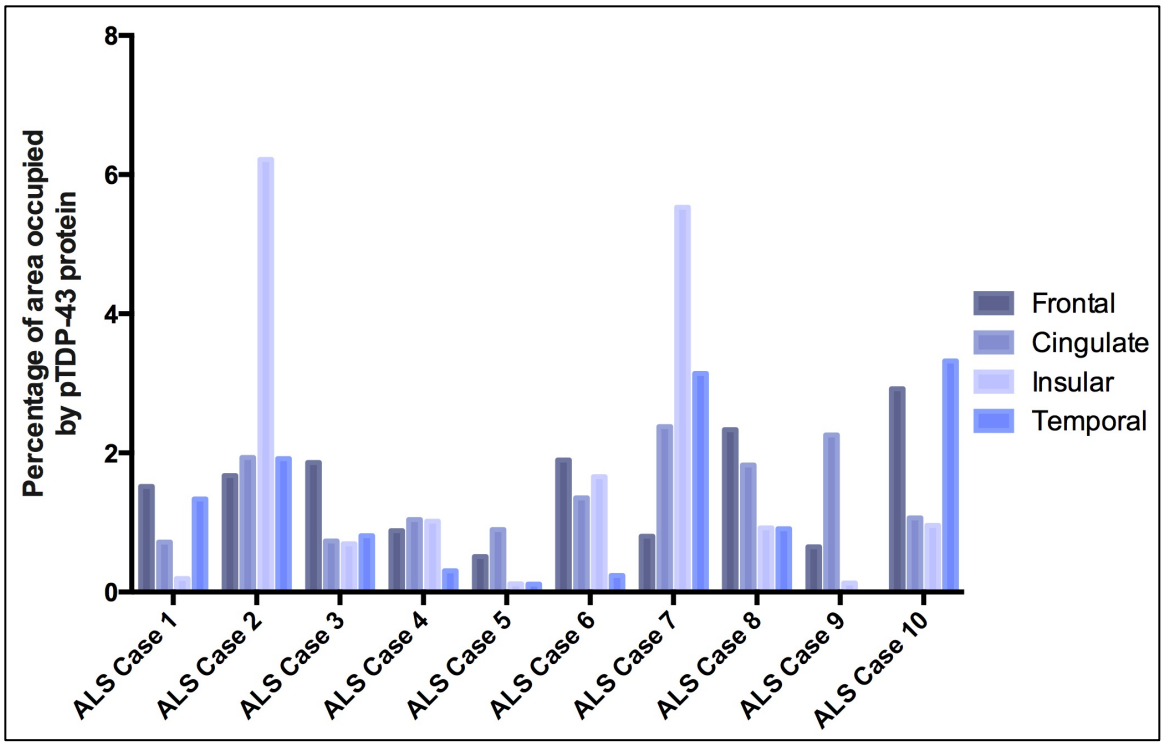

Figure S6 - Qualitative depiction of the cortical area occupied by pTDP-43 by case for ALS Cases 1-10

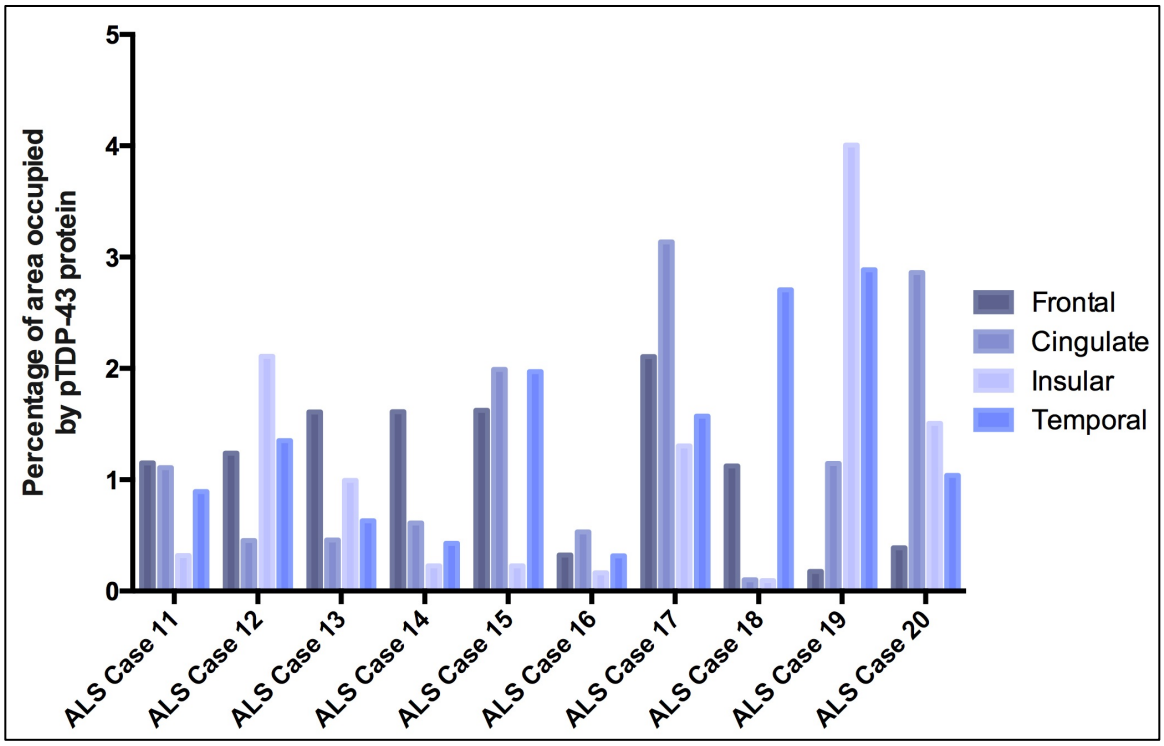

Figure S7 - Qualitative depiction of the cortical area occupied by pTDP-43 by case for ALS Cases 11-20

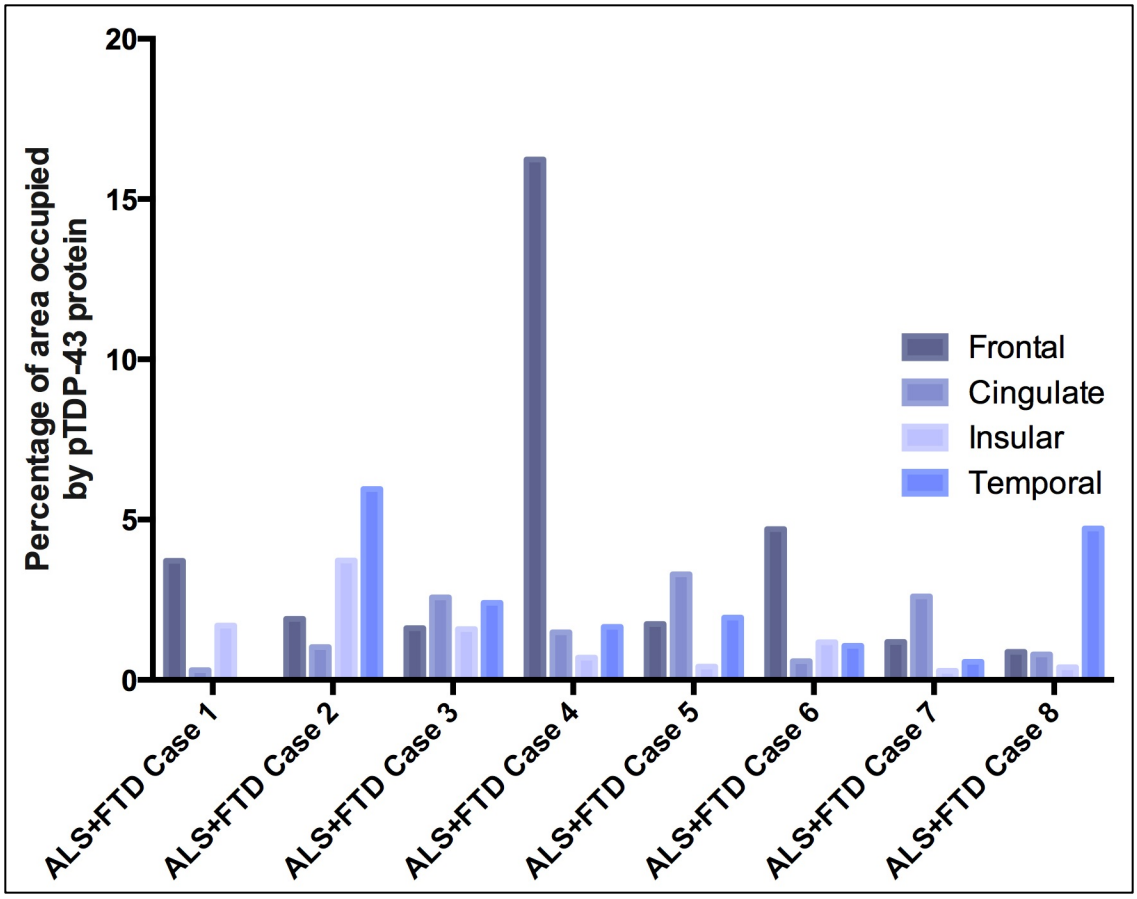

Figure S8 - Qualitative depiction of the cortical area occupied by pTDP-43 by case for all ALS+FTD cases

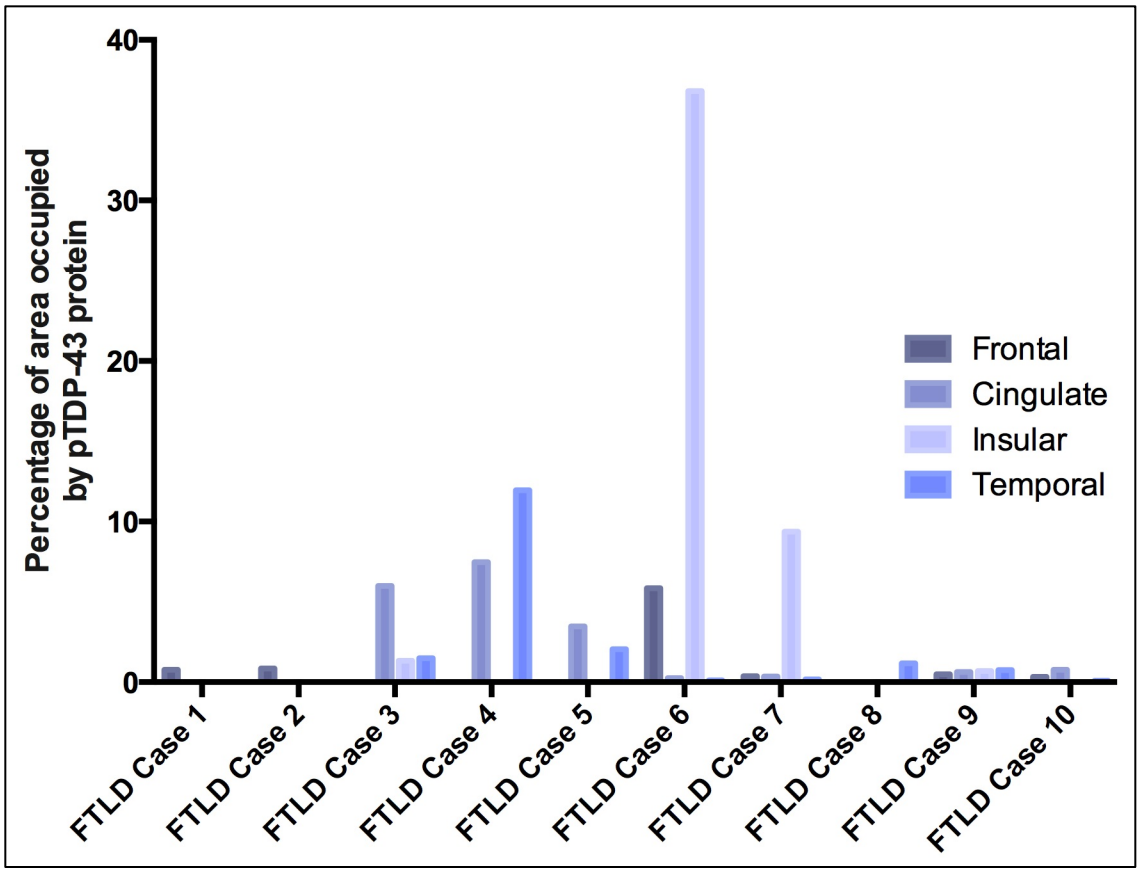

Figure S9 - Qualitative depiction of the cortical area occupied by pTDP-43 by case for FTLD Cases 1-10

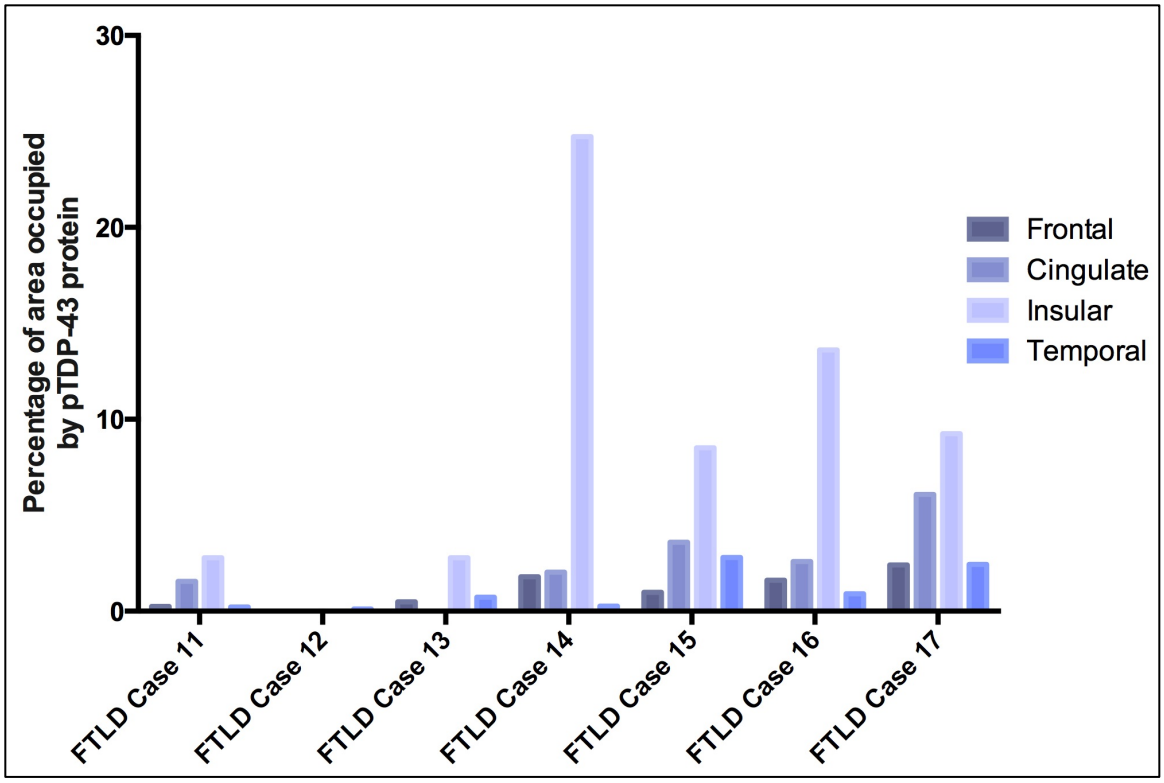

Figure S6 - Qualitative depiction of the cortical area occupied by pTDP-43 by case for FTLD Cases 11-17