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An assessment of possible neuropathology and clinical relationships in 46 sporadic Amyotrophic Lateral Sclerosis patient autopsies

Grant Coan and

Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, USA

Cassie S. Mitchell, Ph.D.

Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, USA

Grant Coan: gcoan3@gatech.edu; Cassie S. Mitchell: cassie.mitchell@bme.gatech.edu

Abstract

Background—Recent studies have suggested overlapping pathological features among motoneuron, cognitive and neurodegenerative diseases.

Aims/Methods—Secondary analysis of 46 Amyotrophic Lateral Sclerosis (ALS) patient autopsies was performed to independently assess pathological feature prevalence (e.g. percent of patients with any positive finding), degree of severity (e.g. mild, moderate, severe), and 2200+ potential clinical/neuropathological correlations. The possible impact of gender, onset age, onset type (limb versus bulbar), riluzole treatment, and severe TDP-43 pathology was assessed within patient sub-groups.

Results—Assessed features (prevalence, severity) include: lateral corticospinal tract degeneration (89%, moderate); Purkinje cell loss (85%, mild); localized neuronal loss (83%, mild to moderate), TDP-43 inclusions (80%, moderate); Betz cell loss (76%, mild); neurofibrillary tangles (78%, severe); anterior corticospinal tract degeneration (72%, moderate); spinal ventral root atrophy (65%, moderate); atherosclerosis (35%, mild); beta amyloid (35%, mild); tauopathy/tau inclusions (17%, mild); ventricular dilation (13%, mild); Lewy body formation (11%, mild); microinfracts (7%, mild); alpha-synuclein (0.04%, mild). Twenty-two percent of patients met criteria for Alzheimer's Disease (AD) and 26% for frontotemporal lobar degeneration (FTLD). Substantive differences were identified in the AD group and in the different onset age groups.

Conclusion—Our findings support the hypothesis that ALS and its variants could comprise a larger neuropathological continuum.

Corresponding Author: Cassie S. Mitchell, Ph.D., Georgia Institute of Technology, 313 Ferst Drive, Atlanta, GA 30332, cassie.mitchell@bme.gatech.edu, 1-404-276-8475.

Keywords

ALS; Amyotrophic Lateral Sclerosis; amyloid beta protein; atrophy; frontotemporal dementia; frontotemporal lobar degeneration; motor neuron diseases; neurofibrillary tangles; tau; Parkinson's Disease

Introduction

In recent years, investigations into the pathophysiology of amyotrophic lateral sclerosis (ALS) have led to suggestions that ALS lies on a pathological continuum with frontotemporal dementia (FTD) [1, 2]. ALS is a disease of the motor neurons most typically characterized by muscle paralysis resulting from the loss of motor neurons in the spinal cord, brainstem, and motor cortex [3]. Nonetheless, mild to moderate cognitive deficits are present in about 45% of the ALS patient population and about 6% develop frontotemporal dementia (FTD) [4].

We hypothesize that a neuropathological continuum could exist that comprises a larger expanse of diseases, including but not limited to: ALS, Primary Lateral Sclerosis (PLS), Alzheimer's disease (AD), frontotemporal lobar degeneration (FTLD), frontotemporal dementia (FTD), Parkinson's Disease (PD), Pick's Disease, among others. The implication that ALS is directly related to diseases whose symptoms comprise, in part, cognitive decline and/or behavioral aberrations, is a departure from the more traditional thought [5], which ascribes to ALS only comprising the aforementioned physical detriment [6]. In addition to the known motor, cognitive, and behavioral impairments, autonomic and homeostatic disturbances have also been reported [7, 8]. Moreover, recent work in the field does indicates that ALS and its variants, such as the Guamian ALS-PD disorder, could potentially exist within a possible etiological spectrum or continuum of multiple neuropathologies [1, 2, 9].

In both clinical and animal model studies, it has become increasingly apparent that many pathology features are shared between various neurodegenerative diseases as well as non-pathological general aging. Such features include protein aggregate inclusions consisting of amyloid beta [10] and/or TDP-43 [11, 12], Lewy bodies [13, 14], and neurofibrillary tangles [15]; neuronal losses and degeneration to specific areas of the brain and spinal cord [3]; circulatory changes of cerebrospinal fluid (CSF) or hemodynamics (blood flow) [16]; and neural atrophy, particularly in the spinal ventral roots, paraspinal fiber, and brain [17]. Such common pathology features could give way to seemingly disparate but yet shared pathological etiologies manifested as a spectrum of different disease phenotypes.

In this exploratory study, we assess possible relationships between clinical and neuropathology features of 46 sporadic Amyotrophic Lateral Sclerosis patient autopsies. We independently assess the prevalence, severity, and correlations between a host of ALS-specific as well as general neuropathology measures in varying locations of the brain and spinal cord, including cellular pathological markers, circulatory and hemoydynamic measures, corticospinal tract degeneration, neural atrophy, neuronal losses of specific cell types and locations, neurofibrillary tangles by location, and pathological post-mortem

diagnosis of co-morbid AD or FTLD. The goal of this study was to identify potentially compelling common pathological features or relationships among this ALS study population that could be statistically evaluated in larger, future studies or compared to other neurodegenerative diseases. In addition to assessing the overall ALS study population, we also examine sub-groups of patients to evaluate the possible impact of gender, co-morbid AD, ALS onset age, ALS disease duration, ALS onset type (i.e. limb versus bulbar), continuous riluzole treatment, and “severe” TDP-43 pathology, on the observed neuropathology features.

Methods

We perform a secondary analysis of autopsy and histological reports completed by a single neuropathologist at Emory ALS Clinic (Emory University Hospital, Atlanta, GA). All post-mortem examinations were performed over a period of approximately ten years. Consent to perform each autopsy for the purpose of research was provided by the patient’s family. Autopsy was performed of the brain and spinal cord for 51 patients of whom 46 patients were included in this study. Examination included a multitude of gross and microscopic parameters as described in Table 1. The internal review boards of Emory University and Georgia Institute of Technology approved this study.

Inclusion criteria

Inclusion criteria consisted of the following: 1) Patients for which the full battery of pathology and immunohistochemisty tests were successfully conducted (see autopsy procedure); 2) Patients with sporadic ALS for which available genetic testing (SOD1, ALS2 gene, etc.) or familial history were negative. Note that given the timeline of autopsies, not all genetic tests (c9orf72, FUS, TARDBP, etc.) were available for all patients; 3) Patients for which complete clinical records were available, including birthdate, sex, first onset symptom date and location, exclusionary diagnostic tests (MRI, CSF, electrophysiology, etc.), riluzole prescription usage history, and genetic testing or complete familial history. These criteria resulted in a total of 46 of the 51 patients being included in the study results.

Autopsy procedure

The brain and spinal cord of each patient was removed and weighed before a sagittal cut. The left half was utilized for present investigation while the right was frozen for future studies. The brain was cut in serial coronal section at 0.5cm interval. Initially, gross examination of the slices was performed examining for pigmentation, atherosclerosis, and visual neurodegeneration. Neuropathology cassette included: hippocampus; amygdala and frontal cortex; parietal and occipital cortex; anterior basal ganglia and caudate; mid basal ganglia and insula; perirolandic cortex; thalamus and hypothalamus; midbrain; pons and cerebellum; medulla and cervical cord; upper cord; lower cord; dorsal root ganglia and dorsal roots; ventral roots. Tissue was fixed in 4–8% paraformaldehyde. Briefly, key immunohistochemistry included: hemotoxylin and eosin (H&E) staining; modified Bielschowsky silver method; TDP-43 antibody; tau anti-body; amyloid beta anti-body; alpha-synuclein anti-body. Pathological feature severity is parametrically determined by extent of staining (see Analysis).

Clinical parameters

Assessed clinical parameters included ALS symptom onset age, onset type, diagnostic delay (the amount of time elapsed between the onset of the first symptom of ALS and diagnosis of ALS by a neurologist), disease duration (the amount of time elapsed between the onset of ALS symptoms and patient death), and prescribed riluzole usage. These parameters were obtained from the Emory University Electronic Medical Records Database.

Neuropathology parameters

Table 1 enumerates and describes the significance of the microscopic and macroscopic pathological features examined in this study. Broadly categorized, they include several different cellular pathological markers; circulatory findings; neural atrophy by location; neuronal loss by specific location or by cell type; presence of neurofibrillary tangles by specific location; and diagnoses of other possible disease in conjunction with ALS, including Alzheimer's Disease (AD) based on pathological CERAD criteria [18] and frontotemporal lobar degeneration (FTLD) based on the post-mortem presence of tau, ubiquitin, or FUS positive inclusions.

Sub-group definitions

Given previously published reports on the impact of onset age [19], suspected Alzheimer's Disease [20], ALS onset type [21], and the presence of TDP-43 inclusions [11] on the ALS neuropathology, we divided the overall population into these aforementioned sub-groups to examine possible differences in their pathological and clinical features.

- Suspected pathological Alzheimer's disease-diagnosis was strictly based on pathological published Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria [18, 22]. Given the 10-year timeline of the autopsies, the CERAD criteria were utilized instead of recent criteria [e.g. 23] to maintain uniformity throughout the study duration.
- ALS onset type-based on recorded clinic survey response of the first symptom location and type, patients were categorized as "limb" (i.e. first symptom appears in muscles of an extremity) or "bulbar" onset (i.e. first symptom appears in the facial muscles or muscles of the throat or tongue).
- Onset age-two separate sub-groups of patients were utilized: 1) patients with an onset age of less than 50 years; and 2) patients with an onset age greater than 60 years.
- Riluzole treatment—patients undergoing continuous treatment with riluzole for the disease duration (from diagnosis through death) based on clinic medical records.
- Severe TDP-43 immunohistochemistry findings: severe TDP-43 classification was based on the extent of staining and corresponding parametric neuropathological feature degree of severity ranking of 4–6 ("moderate") or 7–9 ("severe") described in Analysis.

Analysis

Given the size of the study population, the goal of the analysis was to find potentially interesting or compelling relationships for further research, as opposed to striving for statistical significance. Three different assessments were utilized for each measure: pathological feature prevalence; pathological feature severity; and assessment of relationships between measured pathological features and clinical parameters (age, gender, onset, etc.).

Pathological Feature Pravalence—Pathological feature prevalence is calculated by determining the percentage of patients with a positive finding independent of severity.

Pathological Feature Severity—Pathological feature degree of severity is calculated using the mode of the neuropathologist's severity rating. A parametric severity scale ranging from 1–9 was utilized to quantify the severity of each positive pathological feature (e.g. spinal cord atrophy), with 1 equating to a sparse within-patient presence and 9 representing the most severe presence. Qualitatively, this scale translates to the following relative rankings: 1–3, mild (e.g. sparse or minimal intensity); 4–6, moderate (e.g. marked, pronounced, or persistent intensity); 7–9, severe (e.g. confluent or ubiquitous intensity). The statistical mode (i.e. rating seen the most frequently within a population) of the parametric severity was utilized to assess pathological feature degree of severity.

Pathological Feature Relationships—Relationships between measures are calculated using a cross-correlational analysis of parameterized pathological measures and temporal clinical parameters (i.e. onset age, age at death, etc.). Cross correlation analysis was performed utilizing MathWorks MATLAB software to assess possible relationships using previously published relational analysis methods [24, 25]. A cross-correlation greater than 0.3 was conservatively considered a potentially "promising" relationship worthy of possible further study.

Clinical relationships—Potential clinical relationships between gender, onset age, and disease duration were also independently assessed for the sub-groups shown in Table 2 using a standard student's t-test with $p < 0.05$ signifying possible significance. To insure conservative calculations while using multiple t-test comparisons, standard Bonferroni corrections were applied to the presented results. Normality of the data distributions was determined using the Shapiro-Wilk test. Alternate confirmatory analysis was also performed using the Mann-Whitney test.

Results

The patient population consisted of 46 subjects, including 29 males and 17 females with an overall onset age of 55.8 years with a standard deviation of 12.7 years. Based on the subgroups described in the Methods, of the 46 patients, 10 (22%) met the CERAD criteria for pathological Alzheimer's Disease [18]; 13 patients (28%) had an onset age less than 50 years; 18 patients (39%) had an onset age greater than 60 years; 30 patients (65%) had a confirmed limb onset; 12 patients (26%) had a confirmed bulbar onset; and 29 patients (63%) received continuous riluzole treatment.

Gender, onset and disease duration

We assessed the possible role of gender differences, ALS onset age, and disease duration. Table 2 shows for the overall population, each gender, and for each sub-group, the average onset age and standard deviation and average disease duration and standard deviation. Assessment of gender differences found no *statistically significant* difference between the onset age or disease duration of males and females within the total population or within the assessed sub-groups as noted in Table 2A. Comparison of onset age among the different sub-groups found the following statistically significant differences, as denoted in Table 2B: comparison of the AD versus non-AD sub-groups showed that the onset is later in suspected male AD patients ($p = 0.03$); comparison of the bulbar versus the limb onset showed that onset is later in bulbar onset patients ($p = 0.02$); comparison of disease duration between the different sub-groups found that the male older onset patients (>60 years of age at onset) had a shorter disease duration compared to the male young onset patients (<50 years of age at onset). There were no statistically significant differences in onset age or disease duration for the riluzole and severe TDP-43 sub-groups.

Population prevalence of pathological features

We began our pathological assessment by simply determining the prevalence of each pathological feature in each ALS patient sub-group. The prevalence assessment only takes into account the percentage of patients having a positive finding but does not take into account the feature degree of severity, which is assessed separately. Table 3 lists the prevalence of pathological cellular markers, circulatory measures, neuronal loss by location, neurofibrillary tangles by location, neural atrophy by location, and pathological dementia for the total ALS population and for each sub-group.

Feature prevalence in the total population—Of the pathological markers, TDP-43 was the most prevalent with 80% of the total 46-patient population showing positive inclusions, followed by amyloid-beta (35%), tauopathy (17%) and Lewy body formation (11%). Atherosclerosis was the most common feature of the four circulatory measures with an overall prevalence of 35%. The prevalence of anterior and lateral corticospinal tract degeneration and denervation was high, with prevalence ranging from 67–89%; pigmentary incontinence also had a similar prevalence. Neural atrophy was most prevalent in the spinal ventral roots (65%) and paraspinal muscle fiber (57%). Of the total population, 83% of the patients had a positive finding for neuronal loss in one or more of the 10 assessed locations (see Table 3). The cells or locations with the highest prevalence included Purkinje cells (85%), Betz cells (76%), anterior horn of the spinal cord (43%), and the medulla (33%). Neurofibrillary tangles (NFTs) were present in one or more of the 20 assessed locations in 78% of the total ALS population. NFT prevalence was the highest in the entorhinal cortex (61%), amygdala (43%), nucleus basalis (43%), hippocampus (37%), hypothalamus (22%) and insular cortex (22%).

Feature prevalence in the AD versus non-AD ALS sub-group—Not surprisingly, the AD subgroup had a substantially higher prevalence of amyloid beta, 80%, compared to the non-AD group, 22%. Atherosclerosis and ventricular dilation were also more prevalent in the AD subgroup, but this finding appears to correlate more to age than it does to the

presence of AD (i.e. the AD patients had an overall later ALS onset). NFTs were also substantially more prevalent in the AD sub-group, with NFTs present in 100% of the AD patients. More specifically, the prevalence of NFTs was a factor of 2 higher in the amygdala and hippocampus compared to the non-AD sub-group. Even with the application of an age-matching correction, the prevalence of amygdala NFTs remains a factor of 1.75 times greater in the AD population, which is indicative of an AD-specific relationship.

Feature prevalence in the <50-yr versus >60-yr onset age sub-group—Most notably, the <50-yr onset age sub-group had negligible circulatory findings while the >60-yr onset age sub-group had qualitatively above average prevalence. Most notable in the >60-yr onset age group was the prevalence of atherosclerosis (44%) and ventricular dilation (28%). Additionally, the <50-yr onset age sub-group had a lower prevalence of NFTs both overall as well as in specific locations. More specifically, NFTs were a factor of 2 greater in the amygdala, a factor of 3 greater in the entorhinal cortex, and a factor of 4 greater in the insular cortex in the >60-yr onset age sub-group.

Feature prevalence in the bulbar versus limb onset sub-groups—Pathological markers, tract degeneration, neuronal loss, neural atrophy, and pigmentary incontinence were all very similar between limb and bulbar onset sub-groups. While the overall presence of NFTs was also similar, there were a few potentially notable differences in the prevalence of NFTs at specific locations. For example, the prevalence of NFTs in the hippocampus and nucleus basalis and the prevalence of FTLD was factor of 2 more prevalent in the bulbar onset sub-group.

Feature prevalence in the Riluzole versus non-Riluzole sub-groups—The non-Riluzole subgroup had a small sample size (8 patients). Given the sample size, there are no substantial differences noted in feature prevalence between the Riluzole and non-Riluzole sub-groups. Nonetheless, it is of potential interest that 100% of the non-Riluzole patients had neuronal loss in one or more locations compared to 83% in the Riluzole group and 85% in the total population.

Feature prevalence in the severe TDP-43 sub-group—The TDP-43 sub-group was compared to the total population. Interestingly 100% (26 patients) of the severe TDP-43 sub-group was found to have some form of Purkinje cell neuronal loss as assessed using the modified Bielschowsky silver method. Also potentially notable was that spinal nerve atrophy was a factor of 1.3 greater and FTLD was a factor of 1.5 greater in the severe TDP-43 sub-group. No notable differences were denoted in the prevalence of overall NFTs or localized NFTs.

Severity of pathological features

Next, we examined the severity of the pathological features independent of prevalence. For example, are there some pathological features that are not prevalent (e.g. few patients have a positive finding) but when present, the degree of severity is typically “severe”? In contrast, are there features that are very prevalent (e.g. many patients have a positive finding), but the degree of severity is typically “mild”?

Feature severity in the total ALS population—Of the patients with circulatory findings, the severity was mild. Neuronal losses varied from mild to moderate. Moderate neuronal losses were present in the overall spinal cord, anterior horns of the spinal cord, and the dentate nucleus, while the remaining areas shown in Table 3 had only mild losses. Interestingly, some of the areas of neuronal loss with the greatest prevalence in the patient population had mild severity, including Purkinje cells, Betz cells, and neuronal losses in the medulla. NFT severity varied the most by location, from mild to severe, and was loosely correlated to patient age; interestingly, there was negligible correlation of NFT severity to disease duration. NFTs, when present, were graded as severe in the nucleus basalis, entorhinal cortex, and thalamus and moderate in the locus curuleus, amygdala, insular cortex, anterior horns, and substantia niagra. Pigmentary incontinence, when present, was graded as mild in both the substantia niagra and locus ceruleus. The degree of severity of brain atrophy and spinal cord atrophy were graded equivalently moderate.

Feature severity between sub-groups—For the most part, the overall ALS population and the individually assessed sub-groups shown in Table 3 had equivalent severity ratings for each of the assessed pathological features. However, there were a couple of exceptions in the AD sub-group. More specifically, the NFTs in the amygdala of the AD sub-group were very severe compared to the mild to moderate severities seen in the other sub-groups. Additionally, paraspinal muscle atrophy and Betz cell loss were moderate in the AD sub-group, whereas they were only mild in the other sub-groups. Another substantive sub-group pathological feature degree of severity difference was with onset age. The <50-yr onset age sub-group only had mild to occasionally moderate NFTs with no single location rated at severe; in contrast, the degree of severity of NFTs was typically graded as severe in the >60-yr onset age sub-group.

Relationships between pathological measures or patient sub-groups

As denoted in the Methods, due to the large number of assessed measures and the lesser number of patients in the population and/or sub-groups, statistical analysis was limited to the identification of “promising” relationships rather than traditional statistical significance. Relational and cross-correlation analysis takes into account both prevalence (number of patients with a positive finding) and severity (quantitative degree of positive finding based on the neuropathologist’s rating) to identity possible relationships between two parameters. As sanity checks, we first examined the cross-correlations between known relationships (e.g. cross-correlation between onset age and age at death, $r = 0.98$; paraspinal muscle atrophy and diaphragm atrophy, $r = 0.84$, etc.).

Relationship assessment in the total population—We examined the cross-correlation between each clinical and pathological measure, 2279 possible relationships in total (see supplementary table 1). Key findings connected areas of neuronal loss and neurofibrillary tangles by functional connections in the brain; for example, the hippocampus, amygdala, and entorhinal cortex all had strong relationships for neuron loss and NFTs ($r = 0.5–0.8$). Also, not unexpected, is that circulatory measures were loosely correlated to age, with findings in the range of $r = 0.3–0.4$. TDP-43 inclusions were correlated to Purkinje cell loss ($r = 0.52$) and FTLD ($r = 0.46$). Brain atrophy was most correlated to amygdala NFTs as

well as FTLD (r = 0.47). However, neuronal losses in the brain were most correlated to loss of neurons in the dentate nucleus and medulla (r > 0.5) and to a slightly lesser degree, neuronal losses in the spinal cord, locus ceruleus, and Purkinje cells (r > 0.4).

Assessment of sub-group specific pathological relationships—There were a few substantive sub-group specific pathological relationships. Compared to limb onset, bulbar onset ALS correlated more strongly with anterior and lateral corticospinal tract degeneration as well as patient age at death. Further assessment of upper limb onset versus lower limb onset revealed no notable relationship to any of the examined clinical or pathological parameters. An examination of the relationships with pathological AD did find an expected moderate relationship between amyloid-beta immunohistochemistry (r = 0.5) and hippocampus and amygdala NFTs (r = 0.56). Interestingly, despite the fact that 80% of the overall ALS population had a positive finding for TDP-43 inclusions, there was no notable clinical or pathological relationship difference between those with “severe” TDP-43 inclusions versus “mild” inclusions. Finally, there were no substantive pathological relationships in the cross-correlation analysis that contained gender as a parameter.

Discussion

We assessed the prevalence, severity, and possible relationships between numerous clinical parameters and pathological features from the autopsies of 46 sporadic Amyotrophic Lateral Sclerosis patients. The most prevalent and severe pathological features encompassed TDP-43 inclusions, neurofibrillary tangles, neuronal losses, and corticospinal tract degeneration--all of which were individually identified in greater than 75% of the total ALS study with individual feature degree of severity ratings ranging from moderate to severe. Additionally, our overall results found only minimal impacts of gender, continuous riluzole treatment, onset type, and severe TDP-43 pathology, on discerning differences between these sub-groups and the overall ALS patient study population. However, notable differences were identified in the AD subgroup, less than 50-yr onset age sub-group, and greater than 60-yr onset age sub-groups.

Onset, gender, & disease duration

Our finding that a later ALS disease onset is associated with bulbar onset patients and with patients that appear to have co-morbid Alzheimer’s disease, is consistent with prior work [21]. Also, as has been previously shown, we found that gender does not have a clear impact on clinical patient survival [19], despite reports that female transgenic SOD1 mice tend to have a longer disease duration [e.g. 26, 27]. Further study in larger patient populations is necessary to establish the potential role of gender on clinical ALS parameters.

TDP-43

While other studies have also found that TDP-43 inclusions were ‘common’ in sporadic ALS, even in those patients without actual TDP-43 mutations [11, 12], this is one of the first studies to quantitatively approximate that more than 80% of patients have a positive finding for abnormal TDP-43 inclusions and, of these, the within-patient degree of severity ranges from moderate to very severe.

Betz and Purkinje cell loss

Betz cell loss, which is known to be significant in ALS [28], was found to be very prevalent in this population (76%), although the within-patient severity was typically mild. Similarly, we also found that Purkinje cell loss is a prevalent pathological feature, which has previously only been identified in ALS animal models [29]. Nonetheless, while >80% of patients in this study had Purkinje cell losses, the degree of within-patient severity was typically mild.

Atrophy and degeneration

We also confirm the known correlation between spinal ventral root atrophy and diaphragm atrophy [30] while calculating its pathological prevalence in this population at 65% and 57%, respectively. Finally, we affirm that corticospinal tract degeneration in ALS-FTD is a distinguishing feature that is typically not present in FTD alone [1] and is more strongly correlated to bulbar onset ALS compared to limb onset [31].

Neurofibrillary tangles (NFTs)

There were several interesting findings regarding NFTs. The finding that ALS patients meeting post-mortem pathological AD diagnostic criteria tend to have a higher prevalence and severity of NFTs, especially in the amygdala, hippocampus, entorhinal cortex, and insular cortex, was not necessarily unexpected [32]. However, the 80% prevalence of NFTs identified in the overall ALS study population was notable; moreover, the within-patient degree of NFT severity was typically moderate to severe. Previously, NFTs have mostly been associated with other co-morbid disease such as AD [33], Parkinson's Disease (PD) [34], Pick's disease [35] and other pathologies that include FTD or FTLD [36]. Like the aforementioned study [34], we also found that NFTs were less prevalent in the <50-yr onset age sub-group, and the within-patient degree of severity of NFTs loosely correlated with patient age at death. However, unlike AD-only patients [37], neuronal losses and NFTs did not strongly correlate in ALS patients ($r = 0.22$). Of all the assessed pathological features, it is the entorhinal cortex NFTs, which have the strongest correlation to ALS clinical disease duration ($r = 0.32$). NFTs in the entorhinal cortex and hippocampus have previously been associated with Guamian ALS-PD disorder [34]. While entorhinal cortex NFTs were pervasive in the overall ALS population (61% prevalence, "severe" degree rating), hippocampus NFTs were less so (37% prevalence, "moderate" degree rating). In contrast, entorhinal cortex NFTs and hippocampus NFTs were both simultaneously prevalent and severe in the AD sub-group of ALS patients (80% prevalence, "severe" degree rating).

Based on the overall pathological findings of this study for cellular pathological markers, atrophy, neuronal losses, and NFTs by location, we find that three major functional areas are affected in ALS: 1) motor control (entorhinal cortex, Betz cell loss, Purkinje cell loss, corticospinal tract degeneration, spinal atrophy, ventral root atrophy, etc.); 2) memory, cognition, and behavior (hippocampus, amygdala, beta-amyloid, etc.); and 3) autonomic and homeostatic function (medulla, insular cortex and hypothalamus). Motor control and memory dysfunction are not really a surprising finding. However, autonomic and homeostatic control (including basic body operation, like blood pressure) is less discussed in pathological studies. Nonetheless, autonomic [7, 8], blood pressure regulation [38–41], and

even the potential role of antecedent disease and its affect on homeostasis [e.g. 42], has been previously discussed in the clinical literature. In summary, the widespread identification of overlapping pathological features quantified in this study lends credence to the hypothesis that ALS, FTD, as well as other neuropathologies, could potentially share interrelating etiologies [1, 2, 9], which may represent a larger motor-neurodegenerative-cognitive neuropathological continuum. Clearly, more research is needed to differentiate between disease-specific and shared neuropathological features.

Limitations and Future Directions

As noted in the Methods, given the time span of the study, not all genetic tests were available for all patients. It is possible more recently identified mutations, such as repeat expansions in the C9ORF72 gene [43], could impact the sub-group results. Finally, future similar studies would benefit from newly developed quantitative methods for assessing TDP-43, NFTs, FTLD, and other key markers, which were not available at the initiation of the present study [23, 43–46]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Description of assessed pathology features.

Pathology feature	Description/Reason for inclusion
Beta amyloid	The presence of excess beta amyloid protein, typically in tangles or inclusions, is a known feature of AD [10]. However, beta amyloid changes have also been seen in the CSF of ALS patients [10].
Lewy body formation	Alpha-synuclein immunohistochemistry is used to identify lewy bodies, which are abnormal aggregates of protein present in Parkinson's Disease, lewy body disease, and other disorders, including some cases of ALS [13, 14].
Tauopathy	The presence of tau protein aggregates is a known feature of AD and frontotemporal dementia. The presence of such aggregates in ALS is presently controversial [45, 47–52].
TDP-43 Inclusions	TDP-43, or transactive response (TAR) DNA-Binding Protein 43 kDa [53], is a common pathological feature of both FTD/FTLD and ALS [11, 12]. Cytoplasmic inclusions particularly in the anterior horn, are a common feature of ALS. TDP-43 has been shown to affect neuronal activity response factor in the dendrites of hippocampal neurons suggesting possible roles in regulating mRNA stability, transport and local translation in neurons [54].
Neurofibrillary Tangles (NFTs)	Primarily comprising hyperphosphorylated tau proteins, NFTs are, along with senile plaques, pathological features of Alzheimer's disease. Quantity of NFTs is thought to be related to the degree of dementia, suggesting that their accumulation is possibly related to neuron dysfunction [29, 55]. They have been found in ALS populations in the hippocampus [15].
Atherosclerosis	A specific form of arteriosclerosis in which an artery wall thickens as a result of invasion and accumulation of white blood cells. It has been proposed that ALS could potentially be caused by constrictions in veins draining the spinal cord and brain [56] or from inflammatory disease [16].
Infarcts/microinfarcts	Areas of tissue death due to lack of oxygen. Common in stroke patients. Multi-infarct dementia (MID) can cause a dementia similar to Alzheimer disease (AD). Infarcts/microinfarcts could also be a hemodynamic contributor to ALS [56].
Ventricular dilation	Enlargement of the ventricles may occur for a number of reasons, such as loss of brain volume or impaired outflow or absorption of cerebrospinal fluid. It has been noted in ALS mice as well as some clinical ALS patients [57].
Degeneration of Corticospinal Tract(s)	Corticospinal tracts carry nerve impulses from the brain to the spinal cord, with the majority of fibers crossing at the medulla. Involved in voluntary movement. Both anterior and lateral corticospinal tract degeneration is common in ALS [58].
Denervation	Loss of nerve supply to a given region. Retrograde retraction from the neuromuscular junction is thought to initiate denervation in ALS [59, 60].
Neuronal loss	Neurodegenerative diseases such as ALS are characterized by the loss of certain neurons. ALS, specifically, is characterized by the loss of neurons in regions such as the spinal cord, brainstem, and other areas of the brain, especially the motor cortex [3].
Betz cell (loss)	Large neurons localized to the primary motor cortex. Loss of Betz cells is a feature of motor neuron disease [61].
Purkinje cell (loss)	Large neurons localized to the cerebellum. Loss of Purkinje cells has been implied in ALS. One theory suggests that abnormal trafficking and proteolytic processing of the P2X(4) receptor protein may be involved [29].
Pigmentary incontinence	Caused by external deposits of (typically) intracellular pigments. Is as an indicator of the loss of neurons in pigmented nuclei [62, 63]. We specifically assess the locus ceruleus and substantia niagra, for which pigmentary incontinence has been associated with Parkinson's disease.
Neural atrophy	Shrinking of the neural structure, and more specifically of the ventral roots, diaphragm, paraspinal muscle nerve fiber, and general brain and spinal cord, which have been shown in ALS [17].
Alzheimer's Disease	Neurodegenerative disorder that typically causes dementia. The most common pathological biomarker is amyloid beta plaques or tangles. AD in this study was diagnosed based on pathological CERAD criteria [18, 22].
Frontotemporal lobar degeneration, FTLD	A pathological process that occurs in frontotemporal dementia. Characterized by atrophy in the frontal and temporal lobe of the brain, with sparing of the parietal and occipital lobes. Seen in combination with ALS in some patients [55].

Table 2

Impact of gender, onset, and disease duration. A. Gender, onset age and disease duration for the total study population and for each sub-group. No statistically significant difference in onset or disease duration was found between males and females within any of the sub-groups. Onset age and disease duration are shown as an average in years ± the standard deviation. B. Assessment for statistical significance of gender, onset, and disease duration in the sub-groups.

A.

Sub-group	ALL			MALE			FEMALE		
	N	Onset Age	Disease Duration	N	Onset Age	Disease	N	Onset Age	Disease Duration
All	46	55.8 ± 12.7	4.1 ±2.8	29	55.7 ± 14	4.4 ± 3.1	17	56.1 ± 10.6	3.8 ± 2.1
AD	10	63.6 ± 8.4	3.9 ± 2.8	6	67.2 ± 6.6	3.3 ± 2.3	4	58.3 ± 8.7	4.8 ± 2.1
Non-AD	36	53.7 ± 13	4.2 ±2.9	23	52.7 ± 14	4.7 ± 3.3	13	55.5 ± 11.3	3.4 ± 2.1
<50 yrs	13	40.4 ± 9.3	5.8 ± 3.6	9	39.3 ± 10.7	6.8 ± 3.6	4	43 ± 5.1	3.7 ± 2.8
>60 yrs	18	68.1 ± 4.6	2.6 ± 1.4	12	68.2 ± 4.8	2.3 ± 1.1	6	68 ± 4.5	3.3 ± 1.8
bulbar	12	62.3 ± 13	3.6 ± 3.4	9	61.8 ± 14.1	2.9 ± 3.6	3	63.9 ± 11.7	5.7 ± 1.4
limb	30	53.3 ± 10.5	4.3 ± 2.9	18	52.6 ± 12	5.2 ± 3.2	12	54.4 ± 8.4	3.1 ± 1.7
riluzole	29	55.9 ± 11	3.6 ± 2	18	56.2 ± 12.3	3.3 ± 2.1	11	55.3 ± 9.3	3.9 ± 1.9
non-riluzole	8	58.1 ± 10.3	4.7 ± 3.6	4	57.1 ± 11.1	6.8 ± 3.8	4	59.1 ± 10.9	2.6 ± 2
severe TDP-43	26	57.9 ± 10.8	4.1 ± 2.3	17	58.2 ± 10.6	3.9 ± 2.3	9	57.2 ± 11.7	4.5 ± 2.3

B.

Comparison	All Onset	All Duration	Male Onset	Male Duration	Female Onset	Female Duration
All to AD	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
AD to non-AD	<0.05*	>0.05	<0.05*	>0.05	>0.05	>0.05
Onset <50 yr to >60 yr	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	>0.05
Bulbar to Limb	<0.05*	>0.05	>0.05	>0.05	>0.05	>0.05
Rilutek to Non-rilutek	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
All to sever TDP-43	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Table 3

Prevalence of a positive finding for each pathological feature for the total ALS study population as well as for the sub-groups of Alzheimer's Disease (AD), non-Alzheimer's Disease (non-AD), <50 years of age at onset (<50 yrs), >60 years at onset (>60 yrs), continuous riluzole treatment (riluzole), those who never took riluzole (non-riluzole), and those with severe TDP-43 findings. marker

Assessment	**ALL**		**AD**		**non-AD**		**<50 yrs**		**>60 yrs**		**limb onset**		**bulbar onset**		**riluzole**		**non-riluzole**		**severe TDP-43**	
	%	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**
Sample Size	**100**	**46**	**22**	**10**	**78**	**36**	**28**	**13**	**39**	**18**	**65**	**30**	**26**	**12**	**63**	**29**	**17**	**8**	**57**	**26**
PATHOLOGICAL MARKERS																				
Alpha-synuclein	4	2	0	0	6	2	0	0	6	1	3	1	0	0	3	1	0	0	4	1
Beta-Amyloid	35	16	80	8	22	8	8	1	44	8	33	10	50	6	41	12	50	4	38	10
Lewy Body Formation	11	5	10	1	11	4	8	1	17	3	13	4	8	1	7	2	13	1	4	1
Tauopathy	17	8	10	1	19	7	23	3	6	1	20	6	17	2	21	6	0	0	15	4
TDP-43	80	37	80	8	81	29	85	11	78	14	77	23	75	9	83	24	63	5	100	26
CIRCULATORY																				
Atherosclerosis	35	16	60	6	28	10	8	1	44	8	30	9	33	4	38	11	13	1	38	10
Infarcts	2	1	0	0	3	1	0	0	6	1	0	0	8	1	0	0	13	1	4	1
Microinfarcts	7	3	0	0	8	3	0	0	17	3	3	1	17	2	7	2	13	1	12	3
Ventricular Dilation	13	6	30	3	8	3	0	0	28	5	10	3	17	2	14	4	0	0	23	6
TRACT DEGENERATION & DENERVATION																				
Anterior Corticospinal Tract	72	33	80	8	69	25	69	9	61	11	73	22	50	6	66	19	75	6	81	21
Lateral Corticospinal Tract	89	41	100	10	86	31	77	10	89	16	87	26	92	11	86	25	88	7	92	24
Denervation	67	31	80	8	64	23	69	9	61	11	73	22	67	8	69	20	63	5	69	18
NEURONAL LOSS																				
All (any area)	83	38	80	8	83	30	69	9	83	15	87	26	75	9	83	24	100	8	85	22
Amygdala	4	2	0	0	6	2	8	1	6	1	3	1	0	0	3	1	0	0	8	2
Anterior Horns	43	20	30	3	47	17	23	3	50	9	43	13	58	7	45	13	50	4	46	12
Betz Cells	76	35	80	8	75	27	69	9	78	14	73	22	83	10	72	21	75	6	81	21
Dentate Nucleus	17	8	20	2	17	6	23	3	6	1	17	5	8	1	17	5	13	1	19	5
Gyrus	2	1	0	0	3	1	0	0	6	1	3	1	0	0	3	1	0	0	4	1
Locus Ceruleus	13	6	20	2	11	4	15	2	11	2	13	4	0	0	10	3	13	1	8	2

Assessment	ALL		AD		non-AD		<50 yrs		>60 yrs		limb onset		bulbar onset		riluzole		non-riluzole		severe TDP-43	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
Sample Size	**100**	**46**	**22**	**10**	**78**	**36**	**28**	**13**	**39**	**18**	**65**	**30**	**26**	**12**	**63**	**29**	**17**	**8**	**57**	**26**
Medulla	33	15	40	4	31	11	23	3	28	5	30	9	33	4	34	10	38	3	35	9
Purkinje Cells	85	39	90	9	83	30	85	11	83	15	83	25	75	9	86	25	75	6	100	26
Spinal Cord	24	11	40	4	19	7	31	4	17	3	23	7	25	3	24	7	38	3	31	8
Substantia Nigra	13	6	20	2	11	4	8	1	11	2	10	3	17	2	10	3	0	0	8	2
NEURAL ATROPHY																				
Brain	20	9	30	3	17	6	8	1	28	5	13	4	33	4	17	5	38	3	31	8
Diaphragm	54	25	60	6	53	19	54	7	50	9	63	19	58	7	59	17	50	4	54	14
Paraspinal Muscle Fiber	57	26	60	6	56	20	62	8	50	9	60	18	58	7	59	17	38	3	58	15
Spinal Cord	9	4	0	0	11	4	15	2	6	1	3	1	17	2	10	3	0	0	4	1
Spinal Ventral Nerve Roots	65	30	90	9	58	21	54	7	61	11	67	20	67	8	69	20	63	5	81	21
PIGMENTARY INCONTINENCE																				
Locus Ceruleus	76	35	80	8	75	27	69	9	78	14	73	22	83	10	83	24	63	5	85	22
Substantia Nigra	70	32	70	7	69	25	62	8	67	12	70	21	83	10	72	21	63	5	73	19
NEUROFILBRILLARY TANGLES																				
All (any area)	78	36	100	10	72	26	62	8	83	15	73	22	83	10	83	24	63	5	88	23
Amygdala	43	20	80	8	33	12	23	3	50	9	43	13	50	6	52	15	50	4	38	10
Anterior Horn Cells	4	2	10	1	3	1	8	1	0	0	7	2	0	0	7	2	0	0	0	0
Basal Ganglia	2	1	0	0	3	1	8	1	0	0	0	0	8	1	3	1	0	0	0	0
Caudate Nucleus	2	1	10	1	0	0	0	0	6	1	0	0	8	1	3	1	0	0	4	1
Cingulate Cortex	4	2	0	0	6	2	0	0	6	1	0	0	0	0	0	0	0	0	8	2
Dentate Nucleus	2	1	0	0	3	1	0	0	0	0	3	1	0	0	0	0	13	1	4	1
Entorhinal Cortex	61	28	80	8	56	20	31	4	72	13	57	17	58	7	66	19	63	5	69	18
Frontal Cortex	13	6	10	1	14	5	23	3	11	2	17	5	17	2	17	5	0	0	19	5
Hippocampus	37	17	80	8	25	9	23	3	50	9	27	8	58	7	41	12	38	3	38	10
Hypothalamus	22	10	30	3	19	7	8	1	28	5	17	5	25	3	17	5	38	3	27	7
Insular Cortex	22	10	50	5	14	5	8	1	33	6	20	6	25	3	21	6	13	1	27	7
Locus Ceruleus	30	14	40	4	28	10	23	3	22	4	27	8	25	3	28	8	38	3	38	10

Assessment	ALL		AD		non-AD		<50 yrs		>60 yrs		limb onset		bulbar onset		riluzole		non-riluzole		severe TDP-43	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
Sample Size	**100**	**46**	**22**	**10**	**78**	**36**	**28**	**13**	**39**	**18**	**65**	**30**	**26**	**12**	**63**	**29**	**17**	**8**	**57**	**26**
Neocortex	4	2	0	0	6	2	8	1	0	0	3	1	8	1	7	2	0	0	0	0
Nucleus Basalis	43	20	40	4	44	16	31	4	50	9	30	9	67	8	34	10	50	4	62	16
Occipital Cortex	2	1	0	0	3	1	0	0	0	0	3	1	0	0	3	1	0	0	0	0
Parietal Cortex	2	1	10	1	0	0	0	0	0	0	3	1	0	0	3	1	0	0	0	0
Putamen	2	1	10	1	0	0	0	0	6	1	0	0	8	1	3	1	0	0	4	1
Substantia Nigra	13	6	20	2	11	4	15	2	17	3	10	3	17	2	14	4	13	1	15	4
Temporal Cortex	15	7	10	1	17	6	8	1	22	4	13	4	17	2	17	5	13	1	23	6
Thalamus	15	7	30	3	11	4	8	1	28	5	10	3	25	3	21	6	0	0	19	5
DEMENTIA																				
AD (pathological)	22	10	100	10	0	0	0	0	33	6	20	6	25	3	31	9	0	0	15	4
FTLD (pathological)	26	12	30	3	25	9	0	0	33	6	17	5	42	5	24	7	38	3	42	11