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"Neuropathology of amyotrophic lateral sclerosis and its variants"

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Summary

Amyotrophic lateral sclerosis (ALS) is a clinical syndrome named for its neuropathological hallmark: degeneration of motor neurons in the spinal anterior horn and motor cortex and loss of axons in the lateral columns of the spinal cord. The signature neuropathological molecular signature common to almost all sporadic ALS and most familial ALS is TDP-43 immunoreactive neuronal cytoplasmic inclusions. The neuropathological and molecular neuropathological features of ALS variants primarly lateral sclerosis and progressive muscular atrophy are less certain, but also appear to share the primary features of ALS. A number of genetic causes including mutations in SOD1, FUS, and C9orf72 comprise a disease spectrum and all demonstrate distinctive molecular and neuropathological signatures. Neuropathology will continue to play to a key role in solving the puzzle of ALS pathogenesis.

Keywords

ALS; neuropathology; TDP-43; motor neuron degeneration; C9orf72

Introduction

The first case reports of amyotrophic lateral sclerosis (ALS) date back to Charles Bell in 1824[1]. While a variety of other clinical descriptions followed throughout the 1850s[2-4], the correlations between key clinical features of progressive muscle atrophy and muscle spasticity and key neuropathologic features of loss of anterior horn cells and sclerosis in the lateral columns of the spinal cord were first made by Charcot in the 1860's[5], and thus he named the clinical disease by its distinctive neuropathology.[6] Significant subsequent contributions included the observation of loss of the giant cells of Betz, best summarized by Brodmann in 1909[7], of eosinophilic inclusions now called Bunina bodies in 1962[8], the discovery of ubiquitinated cytoplasmic inclusions in 1988,[9, 10] and the discovery that the

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ubiquitinated inclusions are comprised primarily of TDP-43 in 2006[11, 12]. The association between ALS and frontotemporal lobar dementia (FTLD) has taken three decades to establish[13, 14]. With advances in genetics beginning in 1993, distinctive neuropathology is being identified in the genetic forms, the main ones being SOD1[15], TDP-43[16], FUS[17, 18], and C9orf72 repeat expansions[19, 20].

Classic ALS Neuropathology

Gross

In the majority of ALS brains, no gross abnormalities are observed. The spinal cord often reveals atrophy of the anterior nerve roots[21]. Some cases exhibit atrophy of the precentral gyrus[21]. In patients with dementia, atrophy of the frontal and/or temporal cortex may be seen[21-24], the atrophy being greatest in brains from patients with overlap ALS-frontotemporal dementia (FTD). In addition to these grey matter abnormalities, white matter reduction is also observed, particularly, but not exclusively, in the corticospinal tract[25-27].

Microscopic

Microscopic changes include neuronal and axon loss. There is loss of myelinated axons in the lateral and anterior columns of the spinal cord and decreases in size of anterior horn of the spinal cord, best shown by myelin stains such as luxol fast blue (Figure 1A, B) [21]. There is degeneration and loss of the large motor neurons in the anterior horn of the spinal cord, lower cranial motor nuclei of the brainstem, and Betz cells in the motor cortex, best seen with routine stains such as hematoxylin and eosin (H&E)[21, 28-30] (Figure 1C, D and Figure 2A-F). Morphometric studies of the spinal anterior horn have shown a global reduction of all neurons in the anterior horn, not just the large alpha motor neurons[31]. There is evidence of reduction in neuron size as well as loss and atrophy of nerve fibers. Other pathological features of ALS include vacuolization, large empty spaces near neurons, and spongiosis, microscopic holes resulting in a sponge-like appearance (Figure 2G,H).

Bunina bodies are small (3-6 microns), round to oval shaped eosinophilic intracellular inclusions in the motor neurons of the spinal cord and brain stem of both sporadic and familial ALS patients, best seen with H&E[32,33] (Figure 2I, J). They are most frequently found in the cytoplasm of motor neurons, but can occasionally be found in dendrites[34]. Their number per neuron is highly variable, and they can sometimes make chains and clusters. They are rarely seen in Betz cells, neurons of oculomotor nuclei, and Onuf's nuclei[35-37]. Immunohistochemically, Bunina bodies are positive for cystatin c (Figure 2K, L) and transferrin and partially colocalize with peripherin[38, 39], but they are negative for a variety of proteins commonly associated with neurodegeneration, including tau, alpha and beta tubulin, synaptophysin, amyloid precursor protein, glial fibrillary acidic protein, alpha synuclein, and p62[38, 40-42]. Interestingly, it remains controversial whether or not Bunina bodies are positive for ubiquitin[10, 43]. Their biological significance is unknown.

The complexity of glial cells is now well established and recent studies have shown they are crucial in the biology of ALS neurodegeneration[44]. Reactive astrogliosis surrounds degenerating motor neurons in ALS patients and ALS-animal models[45-47]. Reactive astrocytes show increased immunoreactivity for GFAP and the calcium binding protein

S100β and express inflammatory makers such as COX-2, inducible nitric oxide synthase (iNOS) and neuronal NOS. Increase in GFAP-immunoreactive astrocytes is particularly notable in the grey matter of the spinal cord ventral horn, where normally astrocytes express GFAP at low levels. Cytoplasmic hyaline inclusions and markers of oxidative and nitrative stress accompany astrocyte pathology[48, 49].

Activation of microglia is a critical aspect of the glial neuropathology[45]. It has been correlated with severity of UMN degeneration in ALS (reviewed in Lasiene & Yamanaka, 2011[50]). Activated microglia, responding to neuronal distress, release a variety of proinflammatory cytokines, leading to a higher degree of inflammation in the brains of ALS patients. These proinflammatory molecules include but are not limited to: tumor necrosis factor-α, interleukin-1β, and ionized calcium-binding adapter molecule 2 (Figure 2M, N). Microglia also release reactive oxygen species such as superoxide and nitric oxide, as well as chemokines (monocyte chemoattractant protein 1, macrophage colony stimulating factor) and neurotrophic factors (insulin-like growth factor-1)[51]. It seems neuroinflammation is a two edged sword, some of it protects against neurodegeneration and some of it drives it[44-46].

Molecular Neuropathology: Inclusions and Proteinopathies

Ubiquitin

Some of the most important progress in understanding ALS biology has been driven by key neuropathological discoveries. This began in 1988, when Leigh et al.[9, 52] and Lowe et al.[10] independently discovered ubiquitin-positive skein-like or dense, round structures in the cytoplasm of anterior horn cells in both familial and sporadic ALS (Figure 3 A-D), inclusions that are not detected by H&E and other routine staining methods. Such inclusions were later identified in FTLD and became the cornerstone of distinguishing FTLD with ubiquinated inclusions (FTLD-U) from FTLD with tau (FTLD-tau) and other inclusions [53-55]. In both ALS and FTLD-U, the ubiquitin-positive inclusions have been observed in neurons of the frontal cortex, temporal cortex, hippocampus and striatum[32, 56-59]. Although they are most commonly found in neurons, they have occasionally been seen in glial cells[60]. They are negative for proteins commonly associated with neurodegenerative inclusions, such as tau and alpha-synuclein[56, 60].

TDP-43

The presence of ubiquitin-positive inclusions suggested a problem with some other protein(s). Eighteen years later, TDP-43 was identified as the main component of ubiquitinated inclusions in both ALS and FTD patients[11, 12]. This connected ALS and FTLD-U as TDP-43 proteinopathies[61]. (Table 1) TDP-43 is a heterogeneous nuclear ribonucleoprotein and has many different cellular functions, including mRNA stability[62, 63], mRNA processing[64, 65], mRNA transport and translation[66, 67] and negative regulation of alternative splicing[68]. Under normal conditions, TDP-43 is expressed in many tissues including the nuclei of neurons and glial cells. In sporadic and most familial ALS as well as FTLD-TDP-43 (now renamed from FTLD-U), there is loss of nuclear TDP-43 and formation of pathological aggregates in the cytoplasm (Figure 3E-H)[69]**.** The mechanism behind this redistribution is poorly understood, and could be either the translocation of

TDP-43 from the nuclei to the cytoplasm, or an impaired TDP-43 cytoplasm-to-nucleus shuttling process[12, 70-72]. With the use of immunoblot analysis, extracted material from brains of patients with FTD-TDP-43, FTD-ALS, and ALS were found to be phosphorylated TDP-43 band at 45 kDa[11, 73]. This suggested a post-translational modification to TDP-43. Indeed, antibodies against TDP-43 phosphopeptides stain ubiquitinated TDP-43 positive inclusions in FTD/ALS patients (Figure 3I, J)[74, 75].

There are different kinds of TDP-43 inclusions including fine skeins, coarse skeins, dot-like, and dense round inclusions (Figure 3 E, G, I). Fine and coarse inclusions are seen with a similar frequency in lower and upper motor neurons, while dot-like and round inclusions are seen more frequently in the motor neurons of the anterior horn[76] (Figure 3E-H). In some cases, there is evidence of TDP-43 proteinopathy such as nuclear clearing and/or diffuse or granular cytoplasmic TDP-43 despite the absence of frank cytoplasmic inclusions (Figure 3E)[12, 77].

It is now apparent that TDP-43 inclusions are not pathognomonic for ALS or FTLD-TDP-43, since inclusions are also observed in Alzheimer's disease[78-80], Lewy body diseases[79-82], Guamanian Parkinsonism dementia complex[79, 80, 83] and post-traumatic encephalopathy and neurodegeneration[84]. TDP-43 is present in the mesiotemporal lobe structure in about 30% of the people 65 years or older, regardless of mental illness status[85], indicating the aggregation and misfolding of TDP-43 may be caused by processes normally associated with aging.

Sequential Changes and Neuropathological Staging

Cellular and microscopic

Although it is known that motor neurons degenerate and die in ALS, it is not clear how this degeneration is initiated and progresses or the exact morphological stages of cell death. It is commonly believed that motor neuron death in ALS closely resembles apoptosis, although the evidence for this is incomplete[86]. Martin postulated three stages of neuronal death in motor neurons of ALS patients: chromatolysis (dissolution of the Nissl bodies in the cell body of a neuron), somatodendritic attrition, and finally apoptosis[87]. This process of neuronal death is accompanied by morphologic findings such as cytoplasmic and nuclear condensation and darkness, DNA fragmentation in the presence of DNA fragmentation activation factor, as well as a lack of appreciable vacuolar and edematous cytoplasm in dying neurons. Based on these observations he concluded that apoptosis plays a role in neuronal death in ALS cases. Elevated levels of pro-apoptotic proteins Bax and Bak and decreased levels of anti-apoptotic protein Bcl-2 in vulnerable CNS regions in ALS patients compared with controls were also observed, further strengthening the link between motor neuron death and apoptosis[87]. A related question about ALS regards the spread of the disease and the cell-to-cell spread of disease. A neuropathological correlate of this is that lower motor neuron loss is greatest at the region of onset and decreased outward[8889]. Recently, necroptosis, a form of programmed necrosis involving receptor interacting protein 1 and the mixed lineage kinase domain-like protein, has been postulated to be an important driver of motor neuron death[90], although the neuropathological underpinnings remain to be established.

Anatomical distribution of pathological changes

Staging of ALS neuropathology, similar to Alzheimer's and Parkinson's diseases, has been proposed by Braak et al[91] and Brettschneider et al.[92]. In this, stage 1 disease is characterized by mild burden of pTDP-43 pathology involving motor cortex, brainstem motor nuclei, and spinal motoneurons. Stage 2 disease involves mild-moderate burden of pTDP-43 with dissemination into prefrontal neocortex (middle frontal gyrus), reticular formation, and precerebellar nuclei. Stage 3 disease involves moderate burden of pTDP-43 with dissemination into basal ganglia and prefrontal and postcentral neocortex and striatum. Stage 4 disease involves severe burden of pTDP-43 including the hippocampal formation. In the spinal cord, severity of pTDP-43 pathology in lamina IX motor nuclei and neuronal loss correlated closely with gray and white matter oligodendroglial involvement and was linked to onset of disease[88]. Interestingly, pTDP-43 pathology sometimes included Onuf's nucleus and neurons of Clarke's column but rarely in the intermediolateral nucleus. Gray matter oligodendroglial pTDP-43 inclusions were present in areas devoid of neuronal pTDP-43 aggregates and neuronal loss and suggested involvement is an early event. This staging classification is based on neuropathology, not clinical disease severity (all nervous systems were from patients who died from end-stage disease), and clinical-pathological correlations remain to be established.

Familial ALS: Genetics and Associated Pathology

Approximately 90% of all ALS cases occur sporadically, with no associated family history. The remaining 10% of ALS cases are familial (FALS), and are usually the result of dominantly inherited autosomal mutations. The most common mutations occur in SOD1, TDP-43, FUS, and C9orf72, although several other genes have been identified (reviewed in Renton et al., 2014[93]). The reader is also referred to the chapter titled "Familial ALS" in this issue. Each genetic cause correlates with a relatively distinctive neuropathological signature.

SOD1

Mutations in the superoxide dismutase-1 (*SOD1*) gene account for 20% of all FALS cases. Mutations throughout the gene have been linked to ALS. In general, updating of SOD1 neuropathology is critically needed[105], but it is clear SOD1 ALS patients demonstrate more severe lower motor neuron degeneration than upper motor neuron degeneration. Upper motor neuron degeneration is suggested to be a distal axonopathy[94]. Anterior horn motor neurons also exhibit Lewy body-like inclusions (LBLIs), which consist of a hyalinized, poorly stainable substance (Figure 4A) that by immunohistochemistry are positive for SOD1, ubiquitin, phosphorylated neurofilaments, and various chaperone proteins[96, 97], but negative for TDP-43, p-TDP-43 and FUS[98]. Isotype specific antibodies can uniquely detect misfolded SOD1 in spinal cord motor neurons of patients with *SOD1* mutations (Figure 4B, C). This misfolded SOD1 is absent in the Betz cells in the motor cortex (Figure 4D). Based on the morphology of the motor neurons and the fact that they are TDP-43 negative[95], neuropathology suggest molecular mechanisms of SOD1 mutant FALS may be distinct from sporadic ALS. But, importantly, misfolded SOD1 aggregates have been reported in sporadic ALS as well as mutant SOD1 familial ALS[99-101], thus suggesting SOD1 protein misfolding

may indeed play a role in sporadic disease, although such findings remain controversial[102-104].

TDP-43

The discovery of TDP-43 proteinopathy in 2006 was quickly followed by the identification of mutations in the *TARDBP* gene that encodes it. Mutations are responsible for 2-5% of FALS cases. Approximately 30 mutations have been identified throughout *TARDBP*[106, 107], nearly all of them in the glycine-rich domain, which is responsible for regulating gene expression and protein-protein interactions[108]. Importantly, the TDP-43 and pTDP-43 proteinopathy that are observed in sporadic ALS are also observed in familial ALS caused by *TARDBP* mutations. In a neuropathologic study of patients with the Gly298Ser TDP-43 mutation, inclusions were observed in various locations throughout the CNS, including the substantia nigra, dentate gyrus, cingulate gyrus, amygdala, and the frontal and temporal cortices. The quantity of TDP-43 pre-inclusions in FALS patients with this mutation appears to be greater than in SALS patients[109].

FUS/TLS

In 2009, mutations in the RNA-binding protein fused in sarcoma/translocated in sarcoma (FUS/TLS) in a subset of FALS patients were identified[17, 18]. FUS mutations are responsible for 5% of FALS cases, and are distinct from TDP-43 proteinopathies, but follow a similar motif wherein a protein involved in RNA metabolism is mislocalized from the nucleus and aggregates in the cytoplasm of neurons. Mutant FUS forms large, ubiquitinated, TDP-43 negative neuronal cytoplasmic inclusions (NCI) and occasional neuronal intranuclear inclusions (NII) in the spinal cords and brains of affected patients[110]. These inclusions take the form of fine and coarse granules, as well as filaments, and can be seen in neurons and glia. They are thought to interfere with RNA processing[111] and cause the formation of cytoplasmic stress granules[112]. In contrast with TDP-43 NCIs, cytoplasmic FUS aggregates and non-pathogenic nuclear FUS are not exclusive, and can be observed in the same cell. There is one report that FUS immunoreactive NCIs may be present in sporadic ALS[113], but the specificity of the antibodies was not proven and there has been no further confirmation. Bunina bodies are absent following H&E staining, however basophilic cytoplasmic inclusions are present. Specific FUS-ALS mutations may cause distinctive severity and neuropathology[114]—the p.P525L FUS mutation, with early-onset, has basophilic inclusions and round FUS-positive NCI, while the p.R521C mutation has tangle-like NCI and numerous cytoplasmic inclusions in oligodendroglia. FUS proteinopathy is now understood to account for cases of FTLD-U that are TDP-43-negative[115] and thus cause an ALS-FTLD spectrum[116].

C9orf72

In 2011, abnormally expanded GGGGCC hexanucleotide repeats in C9orf72 were identified as the most common genetic cause of FALS and FTD[19, 20]. This not only linked ALS and FTD at the genetic level, but connected them to the repeat expansion diseases. C9orf72 neuropathology displays the signature ubiquitin-positive, TDP-43-positive immunoreactive aggregates in neuronal cytoplasm, and thus is a TDP-43 proteinopathy (Figure 4E F). But it

is unique among the TDP-43 proteinopathies in several respects. Most of the ubiquitinated inclusions in C9orf72-ALS are p62-positive, but TDP-43-negative[117, 118]. Nucleoporin 62 (p62) is a component of the nuclear envelope and thought to be involved in mRNA and protein trafficking into and out of the nucleus[119]. Another signature is the production of dipeptide repeats proteins (DPRs) resulting from repeat-associated non-ATG (RAN) translation, which occurs bidirectionally. DPRs translated from the sense strand are poly Gly-Ala (Figure 4G,H), poly Gly-Pro (Figure 4I, J), and poly Gly-Arg. DPRs translated from the antisense strand are poly Gly-Pro (also coded by sense), poly Ala-Pro, and poly Pro-Arg. All have been observed in CNS material of C9orf72 cases, although DPRs originating from the sense strand seem to be more frequent than antisense-related dipeptides[120, 128]. DPRs colocalize with p62 but not TDP-43[129, 130]. DPR aggregates can be seen in different parts of CNS including, frontal, occipital, temporal, and motor cortex as well as subcortical areas, midbrain, cerebellum and spinal cord but TDP-43 pathology may not correlate better with disease stage[131]. Another signature of C9-ALS neuropathology is foci of RNA of the expanded repeat (Figure 4K, L). These are detected by fluorescent in-situ hybridization (FISH)[120], and are a feature of several of the repeat expansion diseases[121]. The RNA foci are bidirectionally transcribed and both sense- and antisense-directed expansions are seen[125, 126]. The foci are in multiple cell types including motor neurons, microglia, and astrocytes[20] and in multiple regions of the nervous system including frontal cortex, motor cortex, hippocampus, cerebellum, and spinal cord, as well as lymphoblasts, fibroblasts, and IPSC-derived neurons[122,123, 124]. RNA foci seem to accumulate in cells with TDP-43 protein abnormalities[127].

ALS variants

There are many clinical variants of ALS that appear to be distinctive and a key debate is whether these are distinct disease entities with different biologies, or ends of a continuum. The neuropathological evidence is scarce but suggests the latter, that they share a similar neuropathology and differences are based on the anatomical distribution of the pathological burden rather than biological differences. Separate from this debate about ALS are the important genetic syndromes that affect the motor system but are clearly different from ALS —since these may be confusing, they are reviewed here briefly for clarification.

Primary Lateral Sclerosis (PLS)

PLS is characterized by its upper motor neuron pattern with little or no apparent lower motor neuron involvement.[132, 133,134.] In this issue, a detailed description can be found in the chapter titled “Primary Lateral Sclerosis.” By some estimates, PLS is approximately 0.5% as prevalent as ALS. The most commonly reported differences in neuropathology between ALS and PLS lie in which regions have greater demyelination. PLS reportedly shows the greatest demyelination near the corpus callosum, whereas in ALS demyelination occurs most in the superior frontal gyrus[135]. But, PLS neuropathology does show changes in lower motor neurons and these changes are of the same molecular pattern as is seen in typical ALS disease including TDP-43 pathology, at least in some cases [133,136,137]. Further study is needed to characterize the hallmarks of ALS, including TDP-43 and Bunina bodies, in PLS.

Progressive Muscular Atrophy (PMA)

PMA is characterized by it lower motor neuron pattern with little or no upper motor neuron involvement[138]. The reader is also referred to the chapter in this issue titled “Progressive Muscular Atrophy.” ALS and PMA also share similar genetic mutations in familial cases[139]. Despite the predominant involvement of lower motor neurons clinically, neuropathological studies have shown degeneration of the corticospinal tract even in the absence of upper motor neurons symptoms or signs[140]. PMA neuropathology may show abnormalities of the UMN by way of CD68 staining of the descending corticospinal tract, abnormalities identified in 50% of patients with clinically isolated LMN disease[140]. Distinct pathological change is identified in the motor and extra-motor areas of the brains as well as the spinal cords of patients whose disease was clinically limited to the LMN and these changes seem independent of progression rate[141] Importantly, as is seen in ALS, inclusions positive for ubiquitin, TDP-43, and FUS are frequently present[140, 142]. Thus, the strongest evidence points to PMA being part of disease spectrum, not a different disease. .

Overlap FTD

The clinical overlap of ALS and FTD[143, 144] is mirrored at the neuropathological level. Nearly all sporadic ALS and most FALS (except SOD1 and FUS) show TDP-43 proteinopathy, whereas only about 50% of FTLD is a TDP-43 proteinopathy. Most of the remaining cases of FTLD are considered as tauopathies and a small percentage are FUS proteinopathies. The discovery of repeat expanded C9orf72 revealed a common genetic link between ALS and FTD and highlighted the fact that both ALS and FTD are phenotypes of disease, as well as diseases. C9-ALS and C9-FTD share many pathological markers (as outlined above), and it remains to be shown whether or not C9-ALS, C9-ALS/FTD, and C9-FTD are different neuropathologically—the assumption is that they are not[129]..

Spinal Muscular Atrophy (SMA)

Spinal muscular atrophy (SMA), like PMA, is also characterized by degeneration of lower motor neurons [145] and result from homozygous mutations in the *SMN1* gene[146]. SMA affects infants, juveniles, and young adults and is the leading genetic cause of infant death[147]. A detailed review of SMA is discussed in Chapter 13 of this issue. Affected individuals exhibit muscle weakness and atrophy of muscle fibers[145]. There is also extensive motor neuron loss, gliosis, neuronophagia, and chromatolysis in the anterior horn[145,148]. Reduction in the number of Betz cells in the motor cortex has also been observed[148]. TDP-43 proteinopathy does not appear to contribute to SMA biology, at least in mouse models[149].

Hereditary Spastic Paraparesis (HSP)

HSP is a group of genetically heritable diseases that present with late onset slowly progressive spasticity of the lower limbs. The neuropathological findings are almost entirely limited to the pyramidal tracts of the spinal cord, and most significantly affect the longest ascending and descending axons. This axonal degeneration is particularly noticeable in the lumbar region of the spinal cord. Some degeneration of the anterior corticospinal and spinocerebellar tracts is also observed, as well as occasional loss of the cells in the anterior

horn[150, 151]. Only one mutation of HSP has been studied neuropathologically and did show evidence of TDP-43 proteinopathy [152].

ALS Neuropathology: Future Directions and Final Remarks

The clinical syndrome called ALS is actually named by its neuropathology, amyotrophy and lateral sclerosis. Now, with the rapid progress in our understanding of phenotypes, genetics, and molecular biology and with the availability of new microscopic technologies including immunohistochemistry, immunofluorescence, and in situ hybridization, we are beginning to appreciate the extraordinary microscopic and molecular complexity underlying ALS neuropathology and its importance in unraveling the mystery of disease biology.

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KEY Points

- ALS has a distinctive and complex neuropathology, from which its name is derived.
- Many developments in ALS research have been driven by key neuropathological insights, such as the identification of ubiquitinated cytoplasmic inclusions that led to the identification of TDP-43 in ALS.
- New microscopic and visualization techniques are allowing researchers an unprecedented view of the inner workings of the disease at the gross, cellular, and molecular levels

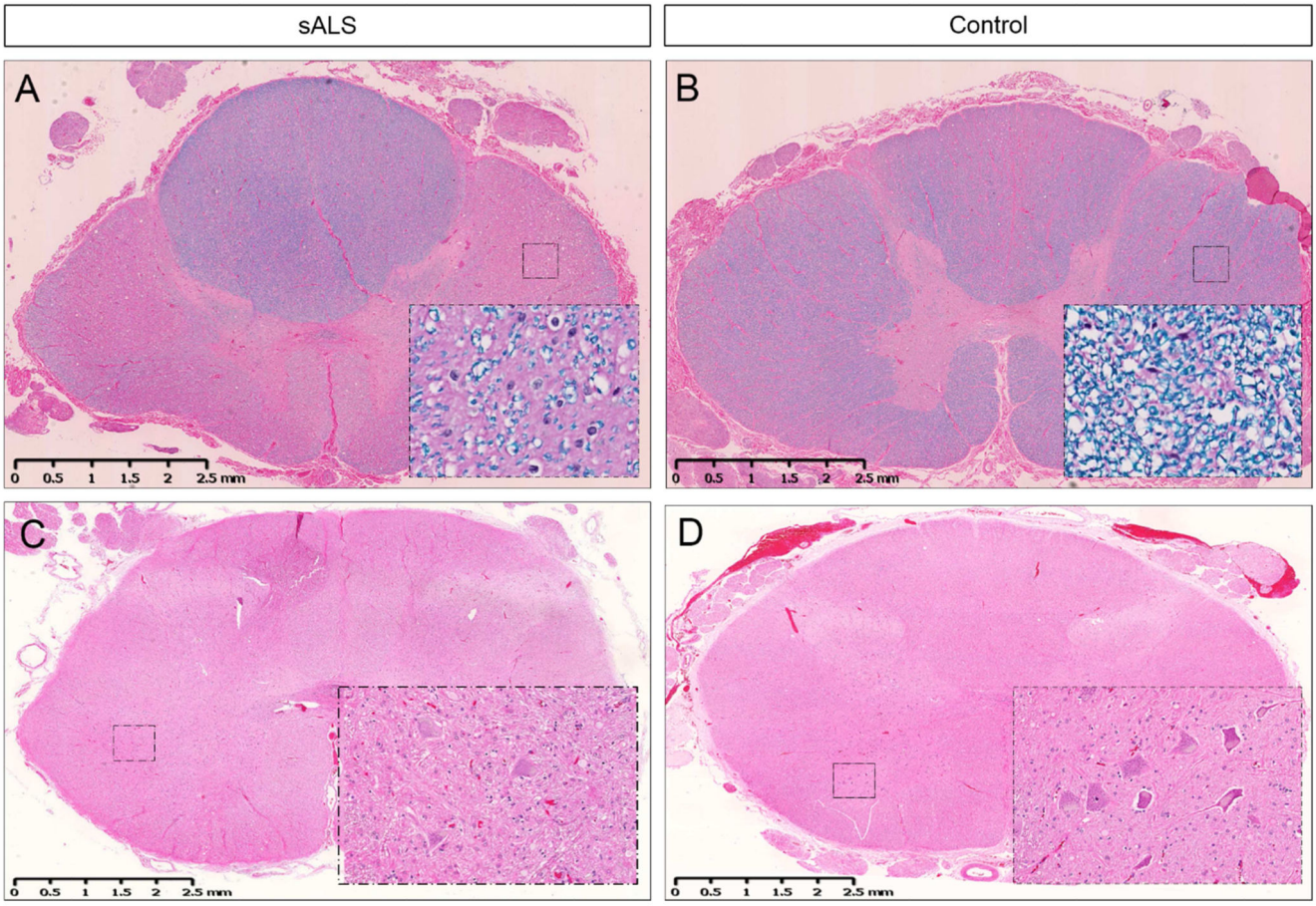

Figure 1. Amyotrophic lateral sclerosis (ALS)

Lateral sclerosis is shown in the thoracic spinal cord in sporadic ALS (A) and compared to control (B). The inserts show loss of myelin in the white matter tracts. Loss of motor neurons is shown in the lumbar spinal cord in sporadic ALS (C) and compared to control (D). The inserts show the motor neurons in the anterior horns under higher power. [Luxol fast blue with hematoxylin and eosin (A, B); hematoxylin and eosin (C, D)]

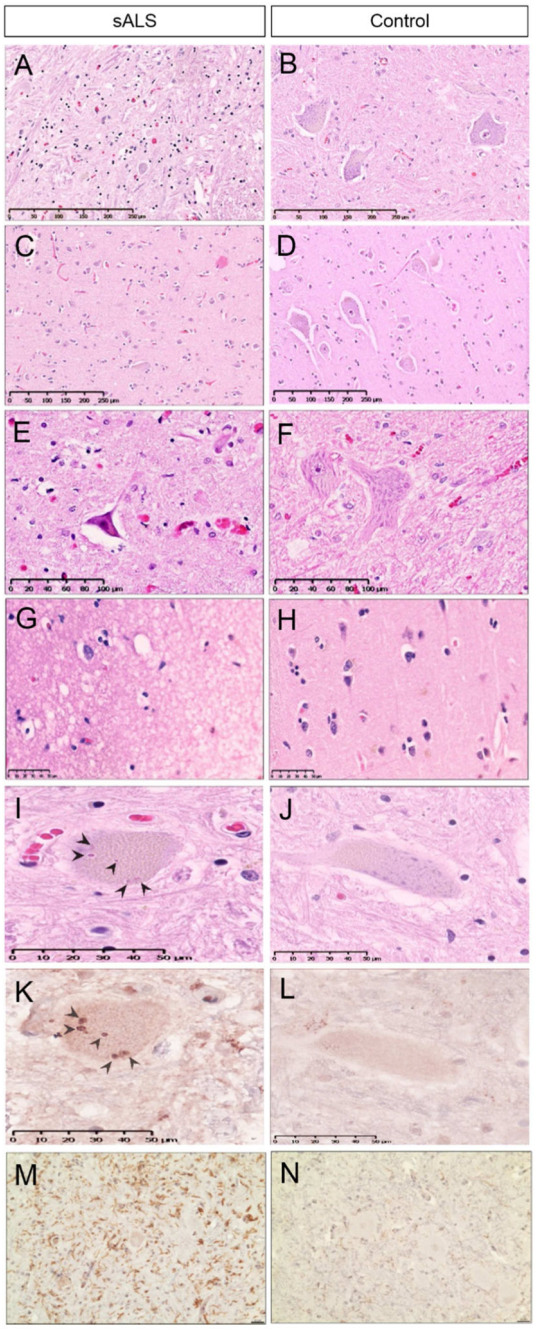

Figure 2. Classic ALS neuropathology

Loss of motor neurons is shown in an anterior horn of the spinal cord (A) and motor cortex (C) of ALS and compared to control (B, D). Shrinkage and contraction of motor neuron in ALS (E) is compared to control (F). Vacuolization and spongiosis in motor cortex is shown in ALS (G) and compared to control (H). Bunina bodies are seen in the cytoplasm of motor neurons of ALS (I) and compared to control (J). Bunina bodies are positive for cystatin c in ALS (K) and compared to the effect of the stain in control (L). Microglial activation is

shown by IBA1 in the anterior horn of the spinal cord in ALS (M) but not control (N). [Hematoxylin and eosin (A-J)]

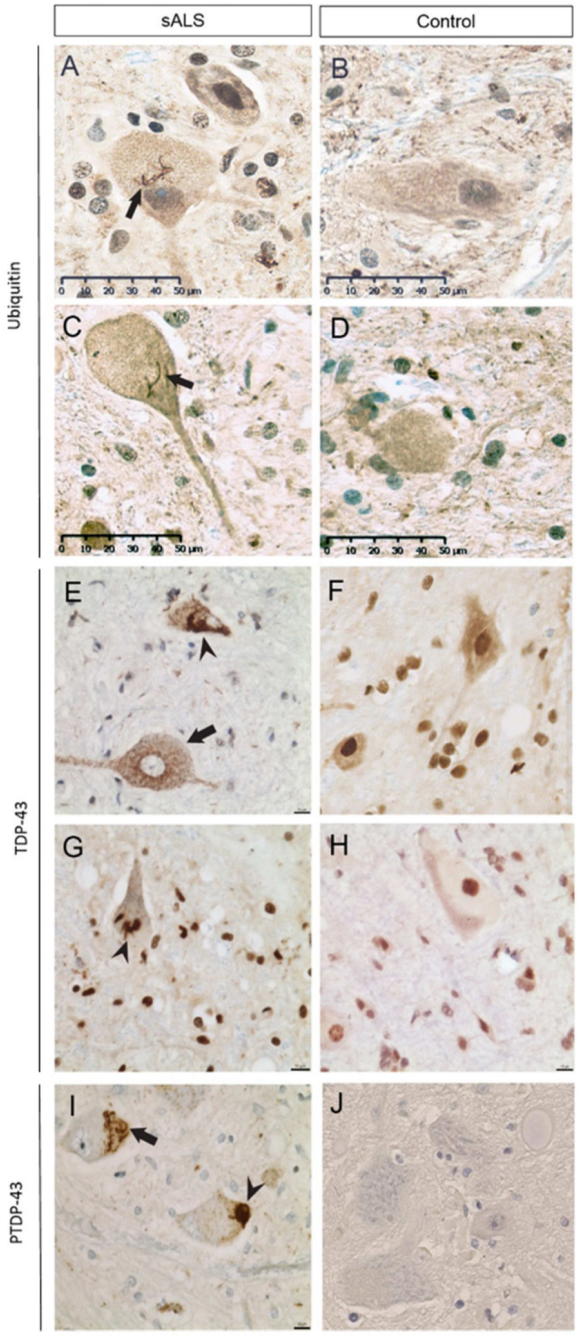

Figure 3. Inclusions in ALS neuropathology

Ubiquitin skein-like inclusions (arrows) are shown in spinal motor neurons of the lumbar anterior horn (A) and Betz cells of the motor cortex (C) in ALS but not control (B, D). TDP-43 inclusions are shown to be diffuse (arrow) and skein-like (arrow-head) and there is nuclear clearing in the spinal motor neurons of ALS (E), features not seen in controls. Note the normal nuclear TDP-43 in control (F). TDP-43 dense round inclusions (arrowhead) are shown in the motor cortex of ALS (G) but not in controls, which show normal nuclear TDP-43 (H). Phospho-TDP-43 staining shows skein-like (arrow) and dense round (arrow-

head) inclusions in ALS lower motor neurons (I), which are not seen in controls. Note pTDP is not seen in normal nuclei (unlike TDP-43) (J).

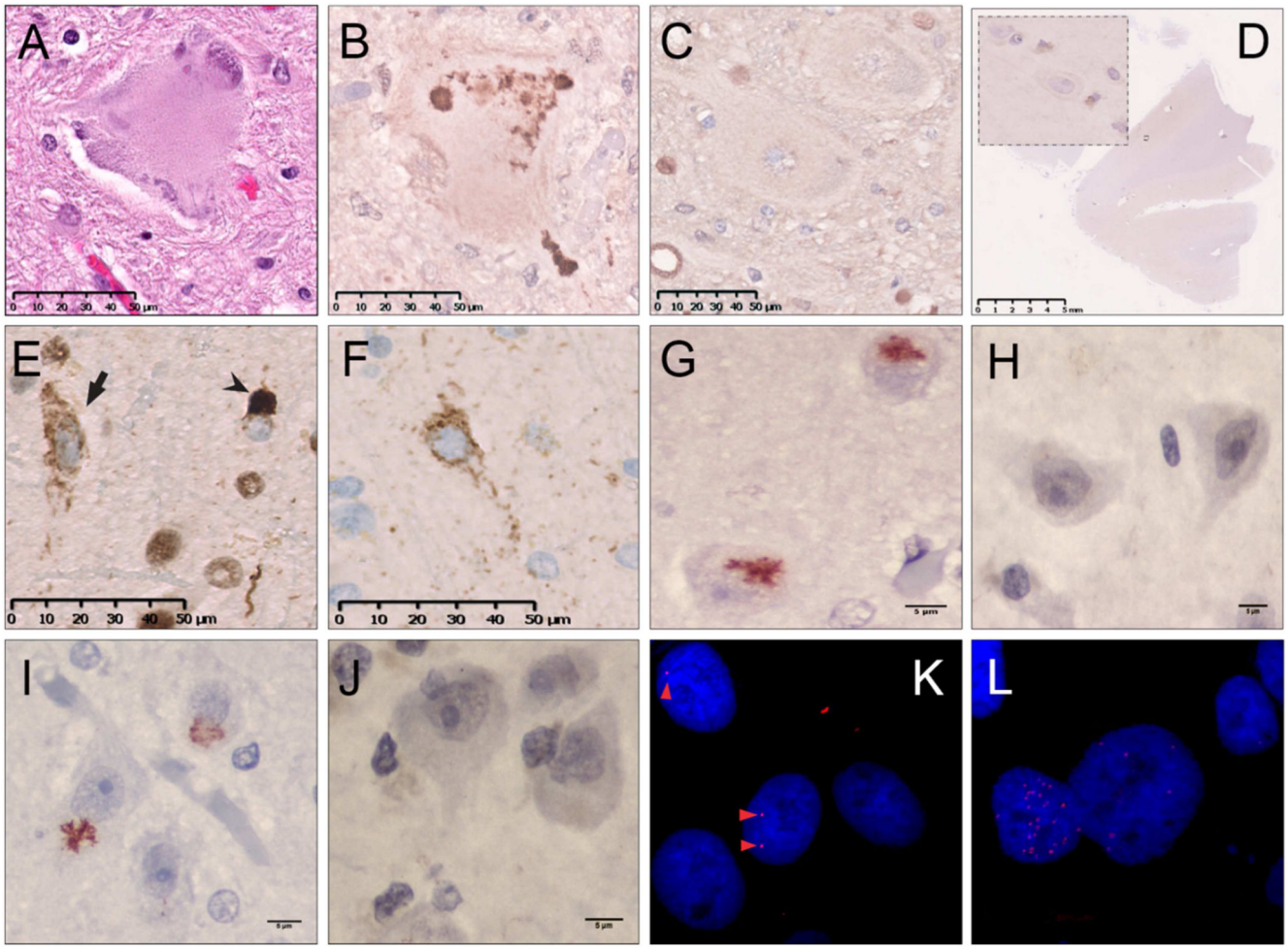

Figure 4. Neuropathology of familial ALS

Lewy body-like inclusion in a spinal motor neuron from a nervous system of a patient with an SOD1 A4V mutation (A). A subsequent histological section showing co-localization of misfolded SOD1 and the Lewy body-like inclusion (B). Misfolded SOD1 is not seen in spinal motor neurons of controls (C) or in the motor cortex of SOD1 patients (D). Skein-like inclusions (arrow) and dense round inclusions (arrow-head) in spinal motor neurons of lumbar spinal cord of a nervous system of a patient with repeat expanded C9orf72 using anti-TDP antibody (E). Same as (E), using an anti-phospho-TDP-43 antibody (F). Poly GA dipeptide repeat proteins in the hippocampus of a nervous system from a patient with a repeat expansion in C9orf72 (G) that are not seen in sporadic ALS (H). PolyGP dipeptide repeat proteins in the hipppcampus of a nervous system from a patient with repeat expansion in C9orf72 (I) are not seen in sporadic ALS (J). RNA foci from the sense (K) and antisense (L) directions from cultured fibroblasts of a patient with repeat expansion in C9orf72 using fluorescent in situ hybridization.

TABLE 1

ALS PROTEINOPATHIES: MAIN MOLECULAR NEUROPATHOLOGICAL FEATURES

Proteinopathy	Phenotype	Gene	Main Molecular Features[1]				
			Motor cortex (UMN)	**Spinal anterior horn or brain stem motor nuclei (LMN)**	**Fronto-temporal regions**	**Miscellaneous (cerebellum, hippocampus)**	**Descending axonal pathways (e.g. lateral columns)**
• **FUS pathology** • **FUS proteinopathy**	• Juvenile ALS • Rare adult ALS (usu. with atypical sxs, e.g. oculo-motility, autonomic, cerebellar or cognitive dysfunction) • FTD	• FUS-TLS	• Basophilic inclusions esp. juvenile cases; • FUS+,TDP43-, NCIs esp. juvenile cases; • FUS+,TDP43-GCIs esp. adult cases	• Basophilic inclusions esp. juvenile cases; • FUS+,TDP43-, NCIs all cases; • FUS+,TDP43-GCIs esp. adult cases	• Rare or none basophilic inclusions; • Rare or none FUS+, TDP43-NCIs; • FUS+, TDP43-GCIs in adult cases	• Rare basophilic inclusions; • FUS+ TDP43-NCIs & GCIs in other regions incl. substantia nigra, nuclei raphe, inferior olives, & dentate nucleus in adult cases	• Degeneration & sclerosis
• **SOD1 pathology** [2]	• ALS, usually LMN predominant features Very rare FTD	• SOD1	• Infrequent abnormalities as seen in spinal anterior horns	• Weakly, ubiquitin+, TDP43-, SOD1-neurofilament+ intracytoplasmic hyaline conglomerates	• Few reports, presumptively same as motor cortex	• Changes also in Clarke's nucleus, dorsal horn, nucleus ambiguous, & Onuf's nucleus	• Distal axonal degeneration • Also, degeneration in dorsal columns
• **TDP43 pathology (non-C9ORF72 related)**; • **TDP43 proteinopathies** • **Ubiquitinated pathology**	• ALS • ALS-FTD • FTD	• Most non-SOD1-associated FALS, including TARDBP • All SALS	• Ubiquitin+, TDP43+ NCIs & GCIs	• Ubiquitin+, TDP43+ NCIs & GCIs	• Ubiquitin+, TDP43+ NCIs & GCIs	• No significant p62+ or UBQLN+ NCIs or GCIs in cerebellum and hippocampus	• Degeneration & sclerosis
• **TDP43 pathology or TDP43 protein-opathies, C9ORF72 variant**	• ALS • ALS-FTD • FTD	• C9ORF72	• Ubiquitin+, TDP43+ NCI & GCI	• Ubiquitin+, TDP43+ NCI & GCI	• Ubiquitin+, TDP43+ NCI & GCI	• p62+, UBQLN+, TDP43- NCIs & GCIs in cerebellum & hippocampus; TDP+ pathology present but separable from p62 & UBQLN	• Degeneration & sclerosis

Proteinopathy	Phenotype	Gene	Main Molecular Features[1]				
			Motor cortex (UMN)	Spinal anterior horn or brain stem motor nuclei (LMN)	Fronto-temporal regions	Miscellaneous (cerebellum, hippocampus)	Descending axonal pathways (e.g. lateral columns)
• **Tau pathology (including FTLD-Tau with Pick bodies)**[3]; • **Tauopathies**	• FTD • Progressive supranuclear palsy • Corticobasal syndrome • Multiple system atrophy	• MAPT	• Signature tau+, ubiquitin-TDP43- NCIs and GCIs	• Few reports, presumptively negative (see 3)	• Signature tau+, ubiquitin-, TDP43- NCIs and GCIs	• Pick bodies = 3R tau+ globular or spherical NCIs in the granule cells of dentate gyrus;	• Presumptively negative

[1] NCI = nuclear cytoplasmic inclusions; GCI = glial cytoplasmic inclusions

[2] No primary FTD phenotypes have been defined by SOD1 pathology.

[3] Included here for comparison–no primary ALS phenotypes have been defined by tau+ neuropathology.