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Amyotrophic lateral sclerosis—a model of corticofugal axonal spread

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Abstract

The pathological process underlying amyotrophic lateral sclerosis (ALS) is associated with the formation of cytoplasmic inclusions consisting mainly of phosphorylated 43-kDa transactive response DNA-binding protein (pTDP-43), which plays an essential part in the pathogenesis of ALS. Preliminary evidence indicates that neuronal involvement progresses at different rates, but in a similar sequence, in different patients with ALS. This observation supports the emerging concept of prion-like propagation of abnormal proteins in noninfectious neurodegenerative diseases. Although the distance between involved regions is often considerable, the affected neurons are connected by axonal projections, indicating that physical contacts between nerve cells along axons are important for dissemination of ALS pathology. This article posits that the trajectory of the spreading pattern is consistent with the induction and dissemination of pTDP-43 pathology chiefly from cortical neuronal projections, via axonal transport, through synaptic contacts to the spinal cord and other regions of the brain.

Introduction

Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset motor neuron disease. It develops rapidly compared with other neurodegenerative disorders (such as Alzheimer disease and Parkinson disease)^{1,2} and is characterized by progressive paresis that leads to death from respiratory failure, with a mean survival of approximately 3 years.³ ALS features inclusion body pathology that consists of ubiquitin protein conjugated with several proteins, among which phosphorylated 43 kDa transactive response DNA-binding protein (pTDP-43) is the most common in individuals with sporadic forms of the disease.^{4–7} Aggregates of pTDP-43 develop in specific nerve cell types and oligodendrocytes of the human CNS. pTDP-43 is, therefore, thought to have an essential role in the pathogenesis of ALS, as well

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Competing interests

The authors declare no competing interests.

Author contributions

H. Braak researched the data for the article. All authors made substantial contribution to discussion of the article content, to writing of the article, and to review and/or editing of the manuscript prior to submission.

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as in other forms of frontotemporal lobar degeneration (FTLD; Box 1), possibly equivalent to that of tau in Alzheimer disease or α -synuclein in Parkinson disease.

Box 1

Phosphorylated TDP-43 pathology in frontotemporal lobar degeneration

- Frontotemporal lobar degeneration (FTLD) is an umbrella term that encompasses a variety of neurodegenerative diseases characterized by predominant destruction of the frontal and temporal lobes.
- Patients with FTLD usually present with frontotemporal dementia, the second most frequent cause of dementia which, in individuals under 65 years of age, has a similar prevalence to Alzheimer disease.
- 43 kDa transactive response DNA-binding protein (TDP-43) was identified as the main component in ubiquitin-positive neuronal inclusions in the largest subset of FTLD, now referred to as FTLD-TDP.⁵ Similar to amyotrophic lateral sclerosis, biochemistry in FTLD-TDP shows TDP-43 to be abnormally phosphorylated, ubiquitinated, and frequently cleaved to generate C-terminal fragments.
- The neuropathology of FTLD-TDP is characterized by ubiquitin-positive and TDP-43-positive neuronal cytoplasmic inclusions (NCIs), neuronal intranuclear inclusions, dystrophic neurites, and glial cytoplasmic inclusions that are negative for tau, α -synuclein, β -amyloid, neurofilaments and expanded polyglutamines.⁵
- FTLD with motor neuron disease-type inclusions but without motor neuron disease (FTLD-U) is classified into four pathological subtypes⁶⁸ that reflect the morphological variability of cortical TDP-43 pathology. For example, cases with the *C9orf72* hexanucleotide repeat expansion—the most common genetic abnormality in familial and sporadic FTLD and amyotrophic lateral sclerosis—are characterized by moderate NCI and few dystrophic neurites across all cortical layers. By contrast, cases with a mutation in the progranulin gene (*GRN*) display NCI and dystrophic neurites, mainly in neocortical layer II.⁶⁸

Emerging evidence suggests that prion-like propagation of altered proteins might underlie the pathophysiology of several noninfectious neurodegenerative diseases. According to this theory, the abnormal proteins could induce a self-perpetuating process that leads to amplification, templating (that is, abnormal protein-induced conversion of natively configured protein to misfolded protein) and propagation of pathological protein assemblies.^{8–16}

Support for the concept that spread or dissemination of pTDP-43 pathology occurs in patients with ALS is derived from clinical observations that progression of ALS is characterized by an increase in the range as well as the severity of motor symptoms over time.¹⁷ Motor manifestations at the time of clinical onset of disease are focal and discrete, and progress contiguously to become diffuse and complex.^{18,19} Indeed, the progression of clinical deficits in ALS seems to be an orderly, sequential and directed process that involves both upper and lower motor neuron levels, with the outward-radiating distribution of both upper motor neuron and lower motor neuron signs preferentially directed to caudal rather than rostral body regions. Symptoms of ALS are more likely to evolve from the bulbar region to the limbs than vice versa.²⁰

In this Perspectives article, we propose that ALS could be primarily a disease of the cerebral neocortex. Formation of pTDP-43 inclusions in neocortical pyramidal cells that generate long axons could represent the primary lesion of this disease, and all resulting lesions in subcortical regions might arise secondarily along corticofugal axonal pathways. We discuss the current evidence for, and potential role and mechanism of, auto-propagation and cell-to-cell transmission of pTDP-43 pathology in ALS via anterograde axonal transport. As ALS and FTLN are clinical manifestations within a pathological spectrum, insights gained from studying the hierarchical sequential propagation of pTDP-43 pathology in ALS could also help to advance understanding of spreading mechanisms in FTLN.

A four-stage model of ALS

Currently, no systems exist to define the stages of ALS on the basis of neuropathology. Recently, our research group has proposed that progression of ALS pathology can be divided into four stages on the basis of changes in the distribution of intraneuronal pTDP-43 aggregates (Figure 1).²¹ Preliminary evidence was gained from staging of 22 CNS regions in an autopsy cohort of 76 patients with ALS, who were classified according to their clinical phenotype and genetic background.

Our findings indicate that the initial lesions develop in portions of the agranular frontal neocortex and in somato motor neurons of the spinal cord and lower brainstem (stage 1; Figures 1, 2).²¹ In the second stage, mild pathology also develops in pre-frontal areas (such as the middle frontal gyrus) as well as in the reticular formation, precerebellar nuclei of the lower brainstem, and parvocellular portion of the red nucleus (Figures 1, 2). In stage 3, the lesions progress into additional prefrontal areas (gyrus rectus, orbital frontal gyri) and then into postcentrally located sensory areas, which is accompanied by the appearance of pTDP-43 pathology in striatal medium-sized projection neurons (Figures 1, 2). In stage 4, cortical pathology also develops in anteromedial portions of the temporal lobe, including the hippocampal formation (Figures 1, 2). Provided the overall burden of pathology remains low, the lesions might have no readily detectable clinical consequences. When they progress within the CNS, however, in what seems to be a systematic manner, cellular dysfunction can exceed a clinical threshold.²¹

Support for the model

The initial insult

Regions of the CNS that are prone to develop ALS-associated lesions are often separated by considerable distances, as is the case with involved areas of the neo-cortex and spinal cord. A notable feature shared by nearly all vulnerable neurons in involved regions, however, is that they receive strong afferents from neocortical pyramidal cells. Moreover, all nerve cells with pTDP-43 inclusions in patients with ALS are controlled and modulated by the cerebral neocortex (Figure 2).

To confirm the directionality of the pathology (that is, whether it involves antero grade or retrograde axonal transport), cellular studies and animal models are required. Nevertheless, our initial findings offer some support for anterograde spread, as virtually all involved subcortical sites in patients with ALS receive strong neocortico-fugal connections (Figure 2).²¹ For retrograde transport along axons, uptake of pTDP-43 (or other molecules) from muscle tissue, the cerebellar cortex, and the pallidum would be needed; however, none of these sites develops florid pTDP-43 pathology during the course of ALS (Figure 2). Furthermore, anterograde transport through cortico fugal axons could conceivably explain how pTDP-43 is transferred from pyramidal cells to neurons at distant CNS sites, resulting in secondary propagation and systematic spread of the pathological process. Such a

pathogenic mechanism would position ALS in a class with other more frequently occurring neurodegenerative disorders, including Parkinson disease and Alzheimer disease, for which neuron-to-neuron propagation via axonal transport has been proposed^{21–24} and confirmed in experimental models.^{25–30}

Neuron-specific susceptibility

Almost all nerve cells that develop ALS-associated pTDP-43 inclusions are projection neurons that generate a long axon, with the exception of affected large cholinergic local circuit neurons in the striatum (Supplementary Table 1 online).³¹ ALS-associated pTDP-43 aggregates seem to be concentrated in motor neurons of the cerebral neocortex, lower brainstem and spinal cord. Nonetheless, the list of vulnerable neurons also includes nonmotor cell types, such as large cortically projecting relay neurons of the thalamus, pyramidal cells in supragranular layers (II–III) of the neocortex, granule cells of the dentate fascia, and pyramidal cells in hippocampal regions CA1–2 (Figure 2 and Supplementary Table 1 online).²¹

Neuronal susceptibility to ALS, therefore, is not confined to motor neurons,^{32–35} and the term ‘motor neuron disease’ does not characterize the pathological process underlying ALS in its entirety.^{36,37} Visceromotor neurons, for example (that is, parasympathetic preganglionic neurons of the sacral spinal cord and of the dorsal motor nuclei of the vagal and glossopharyngeal nerves, as well as spinal cord sympathetic preganglionic neurons), rarely become involved (Supplementary Table 2 online).²¹ In addition, whereas the morphologically uniform group of somatomotor neurons in layer nine of the spinal cord³⁸ and the motor nuclei of cranial nerves XII–X, VII and V exhibit severe pathology in patients with ALS, those of cranial nerves III, IV and VI (which control the extrinsic eye muscles) remain virtually intact throughout the course of the disease (Supplementary Table 2 online).²¹

One reason for the resistance of motor neurons of cranial nerves III, IV and VI to ALS pathology (relative to other cranial nerves) might be their lack of neocortical input: spinal and bulbar somatomotor neurons that become heavily involved in ALS pathology are directly innervated by corticobulbar and corticospinal fibre tracts, whereas control of oculomotor neurons is modulated only indirectly by cortical processes; the major input for motor nuclei of the extrinsic eye muscles comes from noncortical fibre tracts within the medial longitudinal fascicle.³⁹ Similarly, preganglionic visceromotor neurons are chiefly subject to input from noncortical viscerosensory fibres and descending fibres that originate in the gain-setting system of the lower brainstem, which is a system geared to maximizing task-oriented performance and behaviour, particularly under challenging conditions such as novel or stressful situations.^{40,41} The remarkable absence of predominantly cortical control, therefore, could explain why pTDP-43 lesions are so seldom detectable in visceromotor and oculomotor neurons in ALS autopsy tissue.

In this context, the fact that lower brainstem nuclei with diffuse cortical projections (such as the locus coeruleus and upper raphe nuclei) barely become involved in ALS is worthy of note (Figure 2 and Supplementary Table 2 online). By contrast, in other neurodegenerative disorders (Alzheimer disease and Parkinson disease), these nuclei bear the brunt of the subcortical pathology.^{42,43} In other words, sites with projections to the cortex remain intact in ALS, unlike those receiving corticofugal projections (Figure 2).

Sequential corticofugal spread

Analysis of the connections between involved CNS regions in ALS suggests possible routes along which progressive dissemination of the pTDP-43 pathology could occur (Figure 2).

Pathology begins in projection neurons of the frontal agranular neocortex (Brodmann areas 4 and 6) in stage 1.²¹ To date, no reports exist of non-symptomatic patients with ALS in whom involvement is confined to this region alone, but if such patients are described in the future, stage 1 as presently defined by our research group could be subdivided to include lesions limited to the agranular motor cortex only (stage 1a) and agranular neocortical lesions plus pathology in bulbar and spinal cord α -motor neurons (stage 1b).²¹

The initial dissemination of pTDP-43 pathology in stage 1 ALS probably takes place via layer V pyramidal neurons of the neocortical motor cortex. Of note, up to 20% of corticospinal projections in humans terminate directly on α -motor neurons.⁴⁴ For this reason, and because the small interneurons of layer eight (which are mostly upstream from cortical input to somatomotor neurons³⁸) do not develop pTDP-43 pathology in patients with ALS, lesions are likely to spread along axons directly into spinal cord α -motor neurons in layer nine. Dissemination of pathology via short-axon interneurons in layer eight is also improbable because cells with short axons are strongly resistant to the ALS pathological process (Supplementary Table 2 online).²¹

In stage 2, the pathological process in the motor cortex also expands into contiguous portions of the premotor and prefrontal regions and into the bulbar reticular formation and precerebellar nuclei (Figures 1, 2).²¹ A feature shared by the reticular formation and involved precerebellar nuclei is the receiving of major projections from the neocortex,⁴⁵ whereas the other nuclei of this group (which have preponderant input from the spinal cord) either exhibit sparse pTDP-43 pathology or remain intact. The region most frequently involved in stage 2 ALS is the inferior olivary complex.²¹ In humans, however, direct cortico-olivary projections are few;⁴⁶ cortical input to this region comes mainly from cortico-rubro-olivary projections (Figure 2).^{47,48} The ALS pathological process, therefore, could first spread from the cortex to the large parvocellular portion of the red nucleus and then, via the central tegmental tract, to the inferior olivary complex.

During stage 3, involvement of pre-frontal regions progresses. These areas are connected (via longitudinal association bundles) to postcentrally located sensory areas of the parietal, temporal and occipital lobes. Subcortically, pTDP-43 pathology develops in medium-sized projection neurons of the caudate nucleus and putamen (Figure 2).⁴⁹ Each of these nuclei is heavily modulated by descending neocortical fibre tracts.⁵⁰ Interestingly, projections from agranular motor areas terminate in the putamen, whereas those from prefrontal areas are directed towards the caudate nucleus.⁵¹ Axons of striatal projection neurons penetrate the external and internal segments of the pallidum.

In patients with ALS, striatopallidal axons are swollen and contain insoluble particles that are faintly pTDP-43-immunoreactive. The diameters of these axons gradually increase along their lengths and are greatest in medial portions of the internal pallidum where the aggregates are concentrated. Notably, the aggregated material in striatopallidal axons does not induce lesions in pallidal projection neurons (Figure 3). Similarly, no secondarily involved nerve cells are found in the subthalamic nucleus, with the logical result that components of the long pathway through the striatal circuit do not undergo obvious or severe changes in ALS (Figure 2).²¹ Nonetheless, given the existence of the direct route from the agranular motor cortex to the subthalamic nucleus (Figure 2), pTDP-43 lesions might be expected to occur in subthalamic projection neurons. The question of why such lesions do not develop in these cells still awaits an answer.

An additional puzzle in ALS pertains to the involvement of large relay neurons in the thalamus, axons of which preferentially terminate in layer IV of the neocortex. As these are not motor neurons, their development of pTDP-43 inclusions seems surprising (Figure 2).

However, it is conceivable that these large thalamic relay neurons are not inured to the disease process because they also receive strong cortical projections.^{52–54} Moreover, the ALS pathology in thalamic projection neurons fails to spread downstream to the spiny stellate cells of layer IV—possibly because these small pyramidal cells only have short axons, which might render them resistant to the dissemination of pathogenic material.

Via temporal neocortical areas, the cortical pTDP-43 pathology ultimately reaches the transition zone between temporal neocortex and allocortex (transentorhinal region), and involves the adjacent allocortical entorhinal region and hippocampal formation in stage 4 (Figure 2).²¹ The small-celled islands of the pre subiculum, by contrast, remain uninvolved in patients with ALS. For this reason, propagation of the pathological process into the hippocampal formation probably does not take place via pathways through the presubiculum, but instead uses axons that converge from temporal association fields on the entorhinal region, and proceeds from there through the perforant path to the hippocampal formation (Figure 2).⁵⁵

The hippocampal formation itself remains nearly devoid of ALS pathology until late in the disease process—at which point lesions there increase, presumably resulting in a nearly complete loss of hippocampal functions.^{21,56} Notably, granule cells in the dentate fascia are affected more severely than pyramidal cells in hippocampal areas CA1–2. Granule cells are characterized by long and extensively branching axons⁵⁷ and, therefore, belong to the class of selectively vulnerable projection neurons.

The axonal transport hypothesis

ALS-associated lesions are consistently found at sites that receive dense corticofugal projections (Supplementary Table 1 online), whereas sites that project only to the cerebral cortex remain intact (Figure 2 and Supplementary Table 2 online).²¹ Taken together, these observations suggest that anterograde transport of pathogenic molecules via corticofugal axons is essential for propagation of ALS pathological process from neuron to neuron, and that expansion of the pathological process within the cerebral cortex is a driving force behind the progressive regional changes. Although we emphasize here the possible contribution of axonal transport to dissemination of ALS pathology, we can only speculate at this time that the transfer of pathology from upper to lower motor neuron compartments occurs by means of trans-synaptic transmission. The pattern of oligodendroglial pTDP-43 pathology supports the notion of axonal transport as a possible spreading mechanism in ALS: pTDP-43 inclusions mainly occur in oligodendrocytes that have close axonal contact or in those immediately facing axons of diseased neurons, but do not affect satellite cells located along the somata of diseased neurons.²¹

Involved neurons in ALS contain pTDP-43-immunoreactive cytoplasmic material, which can be soluble (at least initially).^{58,59} This material not only fills the entire somatodendritic compartment, but also passes the axon hillock and enters the axoplasm.²⁵ At some point, possibly as a protective measure to neutralize this potentially harmful soluble material, nerve cells produce insoluble dash-like and skein-like pTDP-43 inclusions, which are visible in the axons of bulbar and spinal motor neurons (Figure 3). Remarkably, pTDP-43 inclusions do not, however, develop in corticofugal axons. Biochemical and molecular studies might be able to determine whether the axons of cortical pyramidal neurons contain soluble pTDP-43 (Figure 3).

The absence of pTDP-43 inclusions in corticofugal axons distinguishes them fundamentally from the axons of most other involved nerve cells. If conversion of soluble pTDP-43 into insoluble immunoreactive inclusions could be shown to prevent transmission of ALS-associated pathology to hitherto uninvolved neurons, the main danger would derive not from

the insoluble material in axons of subcortical nerve cells, but from corticofugal axons that still contain reserves of soluble pTDP-43 (Figure 3). Notably, this interpretation favours neuron-to-neuron transfer of pathological material along the axon, rather than cell-to-cell transfection between contiguous nerve cells.^{8–15} If cell-to-cell transfection were the dominant mechanism of transfer, however, the pTDP-43 inclusion pathology would be expected to occur throughout the CNS, instead of in specific types of nerve cells within circumscribed regions.

Summary and outlook

ALS might be primarily a disease of the cerebral cortex. The sequential development of neuronal inclusions in other CNS regions would, according to this viewpoint, be a secondary manifestation of the disease—that is, induced and disseminated by involved cortical projection neurons, along corticofugal axons, for uptake by anatomically distant neurons. Such secondarily involved subcortical projection neurons (for example, medium-sized projection cells of the striatum in Figure 3) that receive input from cortical projection neurons might be able to convert the soluble pTDP-43 in their somatodendritic compartments and axons into insoluble dash-like inclusions, thereby preventing propagation of pTDP-43 pathology to the nerve cells they innervate (for example, projection neurons of the pallidum in Figure 3).

Conversion of soluble pTDP-43 within cortical axons into insoluble aggregates might, therefore, halt progression of ALS by hindering further propagation of the pathological process. Accordingly, one challenge for future research could be to unravel mechanisms that maintain the soluble status of the potentially pathological pTDP-43 material within the axons of cortical pyramidal cells. Another challenge is to identify the conditions that trigger conversion of the noxious (soluble) material into nontoxic (insoluble) inclusions in these axons.

Whether models of ALS can be designed that test the conditions and the spreading hypothesis outlined here remains to be seen. Although models of TDP-43 transmission are in development, animal models that adequately resemble human ALS are needed to confirm the proposed mechanisms of TDP-43 transmission. Transgenic approaches to identify properties of mutant TDP-43 have been reviewed recently.⁶⁰ These models have supported both loss of function^{61–63} and gain of function mechanisms^{64–66} of molecular TDP-43 pathology. Furthermore, they have revealed a crucial role for autoregulatory pathways that maintain TDP-43 RNA levels.^{66,67} These models have considerably advanced understanding of molecular mechanisms of TDP-43 pathology, there are still no animal or cellular models that can reproduce the patterns of TDP-43 pathology observed in human autopsy tissue—most importantly, the combined involvement of upper and lower motor neurons. Development of such models is hindered, in part, by the fact that direct contact between corticospinal projections and spinal cord α -motor neurons is rare in nonhuman primates and other mammals (for example, in macaques, only 5% of corticospinal projections terminate directly on α -motor neurons).⁴⁴ Murine models, therefore, might not fully replicate the spreading of TDP-43 pathology as proposed above.

New models, possibly including non-human primates, could enhance understanding or management of ALS in the future by exploiting anatomical characteristics that underscore neuronal vulnerability. Such studies, in turn, could provide the essential groundwork for strategies designed to interrupt TDP-43 dissemination, which would constitute a fundamentally novel approach to the treatment of ALS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Sequential progression of pTDP-43 pathology in amyotrophic lateral sclerosis. Stage 1: lesions develop in the agranular neocortex and bulbar (XII)/spinal somatomotor neurons. Stage 2: pathology occurs in the reticular formation, precerebellar nuclei, and portions of the thalamus. Aggregates develop in the parvocellular portion of the RN and pars compacta of the SN. Stage 3: the postcentral neocortex and striatum (CN and PU) develop lesions. Stage 4: TE and EN regions, hippocampal area CA1–2 and dentate fascia become involved. Abbreviations: CN, caudate nucleus; EN, entorhinal region; LT, lateral thalamus; MD, medial thalamus; PU, putamen; pTDP-43, phosphorylated 43 kDa transactive response DNA-binding protein; RN, red nucleus; SN, substantia nigra; TE, transentorhinal region.

Figure 2. Corticofugal connections between regions of involvement in amyotrophic lateral sclerosis. Colour intensity indicates the dissemination of pathology during successive neuropathological stages of disease. Grey indicates regions that are not involved. The colour-coded arrows show corticofugal pathways along which the disease process could disseminate, thereby spreading lesions within these relays. Abbreviations: enc, encephalin-containing neurons; sub P, substance P-containing neurons.

Figure 3.

Putative mechanism of pTDP-43 propagation from cortical to subcortical neuronal projections. Initially, soluble pTDP-43 in the somatodendritic compartment of large motor cortex neurons is rapidly converted into dash-like or skein-like aggregates (black), but remains soluble (red) in axons. Intra-axonal soluble pTDP-43 might induce secondary lesions in topographically distant nerve cells that are innervated by these pTDP-43-propagating axons. Discontinuities within axons indicate that they are depicted in an artificially shortened form. Abbreviation: pTDP-43, phosphorylated 43 kDa transactive response DNA-binding protein.