

# ALS motor phenotype heterogeneity, focality, and spread

## Deconstructing motor neuron degeneration



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### ABSTRACT

Heterogeneity of motor phenotypes is a clinically well-recognized fundamental aspect of amyotrophic lateral sclerosis (ALS) and is determined by variability of 3 independent primary attributes: body region of onset; relative mix of upper motor neuron (UMN) and lower motor neuron (LMN) deficits; and rate of progression. Motor phenotypes are determined by the anatomy of the underlying neuropathology and the common defining elements underlying their heterogeneity are that motor neuron degeneration is fundamentally a focal process and that it spreads contiguously through the 3-dimensional anatomy of the UMN and LMN levels, thus causing seemingly complex and varied clinical manifestations. This suggests motor neuron degeneration in ALS is in actuality a very orderly and actively propagating process and that fundamental molecular mechanisms may be uniform and their chief properties deduced. This also suggests opportunities for translational research to seek pathobiology directly in the less affected regions of the nervous system.

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### GLOSSARY

**ALS** = amyotrophic lateral sclerosis; **FTD** = frontotemporal dementia; **LMN** = lower motor neuron; **UMN** = upper motor neuron.

Amyotrophic lateral sclerosis (ALS) is characterized clinically and neuropathologically by deficits of the upper motor neuron (UMN) and lower motor neuron (LMN). The motor phenotypes are well recognized to be highly heterogeneous and determined by 3 primary independent attributes: 1) body region of onset, 2) relative mix of UMN and LMN involvement, and 3) rate of progression. While nonmotor manifestations, especially frontotemporal dementia (FTD), are important, the hallmark of the disease and the reason for regarding it as one nosologic entity is the selectivity for the motor system. That the motor phenotypes are so heterogeneous raises a number of important questions, such as: To what extent, if at all, does heterogeneity suggest variety of molecular mechanisms? How can a single molecular defect (such as SOD1 mutations in familial ALS) produce heterogeneity? In this article, we delineate the relationship between motor phenotypes and the anatomy of underlying neuropathology and we propose common key features underlying the heterogeneity, namely that motor neuron degeneration is fundamentally a focal process and spreads contiguously through the motor system's complex 3-dimensional anatomy. This suggests that fundamental molecular mechanisms in ALS might be uniform, their chief properties predicted, and there are new ways to study them to identify targets for therapy.

**HISTORICAL PERSPECTIVES** Charcot<sup>1</sup> is credited with the earliest descriptions of ALS and he believed that the sclerosis in the lateral columns of the spinal cord induced the loss of neurons in the anterior horns, thus prompting him to name the disease amyotrophic lateral sclerosis and postulating primacy of the UMN. However, he did not discuss the wide variability of UMN and LMN involvement or the focality in early stages of the disease (he actually only examined 20 patients). Gowers,<sup>2</sup> on the other hand, believed that UMN and LMN abnormalities were independent of one another. Most,<sup>3–6</sup> but not all,<sup>7</sup> neuropathologic and neurophys-

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iology studies that have sought correlation between the UMN and LMN have found none, supporting Gowers' view of UMN and LMN independence.

The debate resurfaced in the 1990s when Eisen and others<sup>8,9</sup> postulated that ALS is a disease primarily of the corticomotor neuron, citing the phylogenetic and physiologic uniqueness of the human cortex and postulating an anterograde “dying forward” process in which the UMN recruits the LMN into the degenerative process, thus reformulating Charcot's view of UMN primacy. This model was criticized because of contradicting neurophysiologic and neuropathologic observations,<sup>10</sup> relative lack of testability,<sup>11</sup> and lack of histologic corroboration.<sup>5</sup> Chou and Norris countered by postulating primacy of the LMN and a retrograde or dying-back process triggered by the LMN.<sup>12</sup> More recently, a principal role for the periphery, including skeletal muscle<sup>13</sup> and neuromuscular junction,<sup>14</sup> has been postulated.

Through most of these debates, the uniquely focal manner in which weakness appeared at the clinical onset of ALS and the contiguous outward spread was implied, but not explicitly addressed. Gowers<sup>2</sup> made this clinical observation first, and, indeed, his original description of it is still the best:

... From the part [of the limb] first affected the disease spreads to other parts of the same limb. Before it has attained a considerable degree in one limb, it usually shows itself in the corresponding limb on the other side; often in the muscles corresponding to those in which it commenced . . .

Here he describes onset of motor neuron degeneration in one precise region of the spinal neuraxis, rostral-caudal advancement, and then crossing to the contralateral side. Remarkably, he did this before UMN-LMN motor organization was formally elucidated by Sherrington and before the neural basis of

nervous system function was discovered by Ramon y Cajal.

Most of the clinical literature on ALS focality is descriptive and does not actually analyze the phenomenon.<sup>15-18</sup> Swash<sup>19,20</sup> did analyze it neuropathologically and neurophysiologically and proposed that interaction of multiple factors determines relative susceptibility and resistance for what may be fundamentally a generalized abnormality, thus shifting but not negating the observations of focality. Brooks,<sup>21</sup> in the early 1990s, longitudinally tracked symptom accrual and showed anatomic contiguity, which suggested a role for axonal transport and transneuronal propagation; however, his method involved serial patient questionnaires and thus tracked functional rather than UMN and LMN anatomic changes. Munsat<sup>22</sup> studied the natural history of ALS spread, noting linear uniform progression in different regions of the same patient, but marked variability between patients. Armon<sup>23</sup> observed but did not directly analyze spread, which he hypothesized resulted from certain toxic subcellular components, involving acquired DNA mutations. In 2007, one of us performed a retrospective cross-sectional analysis of initial clinical motor deficits and showed that in early disease there is a linkage between UMN and LMN degeneration in terms of the innervated body region that first manifests motor deficits, but a dichotomy between them for relative severity of involvement and outward spread.<sup>24</sup> This was supported by a post-mortem study of the LMN, which found that the degree of motor neuron loss was related to the site of onset.<sup>25</sup>

### ALS INVOLVES THE COMPLEX 3-DIMENSIONAL ANATOMY OF THE MOTOR SYSTEM

The motor system is distinctive for its 2-tiered organization into UMN and LMN levels and complex 3-dimensional anatomy (table). UMN, or giant cells of Betz, spread as laminar sheets in layer V of M1 (~Brodmann area 4) of the cerebral cortex, organized somatotopically lateral to medial over a distance of 12 cm. LMNs, or alpha motor neurons, stack in columns in brainstem motor nuclei and spinal anterior horns, organized somatotopically rostral to caudal over a distance of 45 cm. During development, neuron progenitors are continuous with each other in the neural tube initially and then separate into 2 levels by anterior-posterior folding, which occurs simultaneously to radial migration and differentiation. UMN and LMNs functionally integrate through a number of physiologic networks. The motor unit, which is composed of the LMN and its muscle fibers, is now well understood.<sup>26</sup> The next best understood are the integrated UMN-LMN networks, which connect by

Table	Comparison of UMN and LMN 3-dimensional neuroanatomy	
Anatomic feature	UMN	LMN
Location	Cerebral cortex	Brainstem and spinal cord
Motor neurons	Giant cells of Betz	Alpha motor neurons
Nuclei	M1 (~Brodmann area 4)	Motor nuclei and anterior gray horn
Microenvironment	Layer V	Rexed lamina IX
3-D arrangement	Laminar	Columnar
Somatotopic arrangement	Lateral to medial	Rostral to caudal
Anatomic span	12 cm per hemisphere	46 cm midbrain to sacral cord
Origination in neurodevelopment	Anterior (rostral) portion of neural tube in line with LMN progenitors	Posterior (caudal) portion of neural tube in line with UMN progenitors
Functional integrations	Prefrontal networks; convergence and divergence with LMNs	Convergence and divergence from UMN; motor units

UMN = upper motor neuron; LMN = lower motor neuron.

convergence—many UMNs innervating one LMN—and divergence—one UMN innervating many LMNs.<sup>27</sup> The least understood are the premotor neuronal networks.<sup>28</sup> The highly selective involvement of ALS for this entire system—LMN, UMN, and premotor—thus suggests that ALS is a motor system rather than a motor neuron disease.

#### CLINICAL EXAMINATION IS STILL BEST FOR ASSESSING NEUROPATHOLOGY IN VIVO

Motor phenotypes in ALS are attributable to the superimposition of motor deficits occurring simultaneously at the UMN and LMN levels and thus reflect the in vivo anatomy of underlying neuropathology. A number of sophisticated neurophysiology techniques assess motor neuron function. The main one for UMN function is transcranial magnetic stimulation of the cortex<sup>29</sup>; the main one for LMN function is conventional EMG,<sup>30</sup> although a plethora of other techniques including single fiber EMG, macro-EMG, scanning EMG, and motor unit number estimates also do this. But for the assessment of the overall anatomy of underlying neuropathology, they are problematic—they are difficult to perform for all body regions, they are difficult to perform serially, and they are not uniformly matched in sensitivity or reliability at the 2 levels. In short, they assess neuron function, not anatomic distribution of neuropathology. Remarkably, little study has actually been devoted to indexing function to topography of pathology.

A number of radiographic imaging techniques assess in vivo neuropathology of the UMN.<sup>31</sup> Conventional MRI including FLAIR, T2-weighted, and proton density images may reveal abnormalities, but they lack both sensitivity and specificity, and do not correlate well with clinical status. Diffusion tensor imaging and a related technique, diffusion tensor tractography, reveal changes in white matter tracts and thus image axonal, not neuronal, pathology; results thus far have been contradictory and studies of early disease have been scarce. Voxel-based morphometry identifies both cortical and subcortical degeneration, but it has inherently low spatial resolution. Functional magnetic resonance spectroscopy, using either neuronal markers, such as *N*-acetylaspartate, or glial markers, such as myo-inositol, image functional aspects of in vivo anatomic pathology, but measurements are variable and serial changes are uncertain.

Thus, despite major technological advances in neurophysiology and neuroimaging,<sup>32</sup> clinical assessment by way of the traditional clinical examination remains the best way to localize neurodegeneration in vivo and to follow the process in real time. It is

reliable, comprehensive, efficient, and uniform at both levels simultaneously—its value cannot be underestimated.

#### FOCALITY AND UMN/LMN DISTRIBUTION IN ALS SHOULD GUIDE HOW WE THINK ABOUT DISEASE MECHANISMS

In many respects, the onset and first clinical manifestations of ALS are the most informative, because deficits and neuropathology have not undergone temporal-spatial summation and are relatively uncomplicated. Numerous studies suggest the following<sup>15-18,21,24,33,34</sup>: 1) focality of initial symptoms is commonplace, occurring in most patients; 2) onset site is randomly localized in the neuraxis; 3) both UMN and LMN deficits are maximal in the same peripheral body region; 4) both UMN and LMN deficits are highly variable in their severities of involvement; and 5) both UMN and LMN deficits spread regionally outward along their independent neuroanatomy. While patients are often classified by site of onset (such as bulbar, spinal, polyneuritic, flail arm, bi-brachial, hemiparetic, or truncal) or by UMN/LMN mix (such as pseudobulbar and bulbar palsy, or primary lateral sclerosis and progressive muscular atrophy), in fact, these distinctions are based upon evaluation of predominant clinical aspects that exist on a continuum. Distinguishing between ALS outliers and other disease entities remains subjective, as arbitrary distinctions between UMN predominant ALS and primary lateral sclerosis<sup>35</sup> or between LMN predominant ALS and progressive muscular atrophy<sup>36</sup> exemplify. In this regard, focality and discreteness of onset may ultimately prove to be as important as any feature for assigning nosology. It is also worth noting that the current classification of FTD, which is now believed to broadly overlap with ALS, into behavioral variant FTD, progressive nonfluent aphasia, and semantic dementia is based upon the anatomy of underlying neuropathology and reflects a fundamental focality of disease onset.

These clinical findings reveal key themes that must be considered when formulating hypotheses about disease pathogenesis: 1) Progressive motor neuron degeneration may have a highly discrete onset. 2) The site of onset may correlate with neuron numbers; this, in turn, suggests that the initial trigger is stochastic (or statistically occurring) at the molecular level—note that “randomness” is emerging as an important concept in molecular biology, as the complexity and fidelity of molecular and cellular processes are being modeled mathematically.<sup>37</sup> Stochastic events, to give one example, could involve variation in local blood flow—one could envision an ill-timed or ill-placed vascular hypoperfusion event triggering molecular pathology mediated by “angion-

eurins” such as VEGF,<sup>38</sup> which are increasingly recognized to play a role in neurodegeneration, thus providing a model for the apparent random, but focal, initiation. 3) The triggering pathogenic event may occur at any level of the motor network (UMN, LMN, or periphery) and be distributed (but not caused) by transneuronal signaling or axonal transport. It is important to point out that there are 2 types of transneuronal signaling. One type is synaptic between neurons in series such as UMNs and LMNs or neurons and interneurons. Synaptic signaling is either retrograde or anterograde and is relevant to the trigger and initial distribution between UMNs and LMNs. The other type is local between neurons in parallel such as neurons proximate to each other at the same anatomic level. Local neuronal signaling may be nonsynaptic and involve the neuron microenvironment and is relevant to local progression and contiguous spread at the respective levels once degeneration is triggered and distributed. 4) Since the severity of involvement at the UMN, LMN, and prefrontal levels is highly variable, distribution of pathogenic (or protective) factors in the motor system must also be stochastic—that is, the degree to which the UMN or LMN levels are affected in a particular patient is determined by a random distribution of an effective trigger throughout the connected UMN and LMN network.<sup>37</sup>

#### **CONTIGUOUS SPREAD: ANOTHER DEFINING FEATURE OF ALS WITH IMPLICATIONS FOR DISEASE MECHANISMS**

A distinctive clinical aspect of ALS is the unique spread of symptoms to contiguous anatomic regions over time. This has been demonstrated by longitudinal<sup>21,22,39</sup> and cross-sectional<sup>24</sup> analyses. Cross-sectional analysis, in particular, suggested the underlying motor neuron degeneration spreads along respective UMN and LMN anatomy, and that over time, this summates temporal-spatially both within and between the UMN and LMN levels, producing increasingly complex phenotypes of motor deficits that ultimately appear diffuse and symmetric. Interestingly, the outward spread of both UMN and LMN signs seems to be weighted toward caudal body regions over rostral ones, and may thus have directionality. For example, symptoms are more likely to evolve from the bulbar region to the limbs than vice versa.<sup>21,24</sup> This suggests that underlying motor neuron degeneration has preferential directions of outward spread, rather than simple radial or centrifugal directions, and that there may be differences in motor neuron vulnerability, a possibility previously raised by Swash<sup>19,20</sup> and by Brooks.<sup>21</sup>

Importantly, the outward spread of motor neuron degeneration at the UMN and LMN levels is along

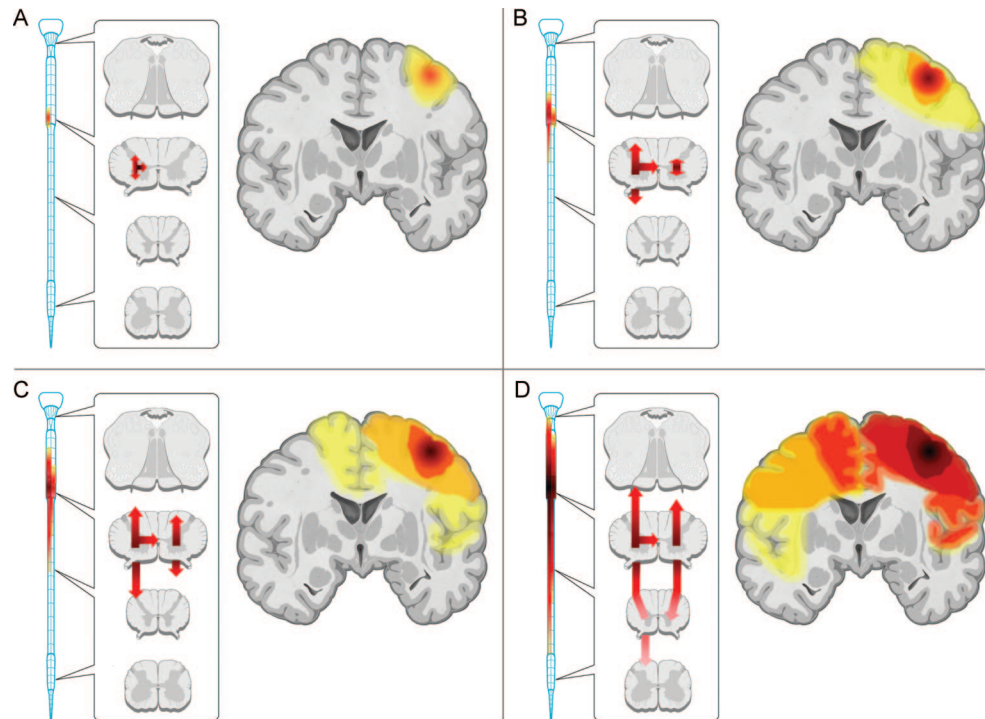
differing and complex 3-dimensional anatomy, as outlined above, and this causes UMN and LMN deficits to appear incongruously, thus accounting for the complexity of motor phenotypes<sup>24</sup>: UMN and LMN clinical deficits can spread to different peripheral body regions because of their differing somatotopic anatomy; or they can spread to the same peripheral body region, but at different times, because of the differing distances of their anatomic spans. This is best illustrated in outward spread from the hand/arm areas (figure), where UMN and LMN differ in both somatotopic organization and spread distances: at the LMN level, spread is first to the contiguous contralateral hand/arm areas and subsequent spread to the ipsilateral foot/leg is far remote through the long thoracic span (about 26 cm). At the UMN level, spread is first to the contiguous ipsilateral foot/leg and subsequent spread to the contralateral hand/arm is only slightly remote (about 3–6 cm). This anatomic explanation idealizes what obviously has greater complexity, with one example of the complexity being pseudobulbar palsy, where UMN degeneration is bi-focal.

Nonetheless, contiguity of spread identifies some of the most critical features that must be considered in hypotheses of disease pathogenesis: 1) ALS is an active as opposed to passive process (murder rather than death)—perhaps analogous in concept to the toxic gain (as opposed to loss) of function for mutant SOD1; 2) it is a propagating process that recruits locally; 3) it is nonaccelerating; and 4) it is orderly. A number of fundamental molecular mechanisms could explain this: these include but are in no way limited to signaling factors such as cytokines, chemokines, or other paracrine signals; aberrant transmembrane signaling pathways; diffusion of a toxic fraction through the neuron microenvironment; role of non-neuronal cells, especially glia; or protein folding. Consistent with this prediction, astrocytes expressing mutant SOD1 protein have been proposed to secrete a toxic factor that selectively injures motor neurons.<sup>40</sup> Directionality of spread may involve variability in motor neuron susceptibility, perhaps due to differences in size, axon length, dendritic arborization, gray matter position, or microenvironment.

#### **VARIABLE PROGRESSION RATES: FACTORS CONTROLLING DISEASE KINETICS**

To the 2 anatomic determinants of motor phenotype—site of onset and UMN/LMN mix—a third and dynamic determinant should be added: progression rate. Progression rates in ALS are usually linear for any one individual patient, but are highly variable among different patients, ranging from rapid (1 year) to slow (>10 years).<sup>22,41,42</sup> Progression rate reflects the rate of

**Figure** An idealized model of the natural history of amyotrophic lateral sclerosis (ALS) based upon focality and contiguous spread



(A) Onset: At clinical onset, degeneration involves upper motor neurons (UMNs) and lower motor neurons (LMNs) that innervate the same peripheral body region; the site of onset, ratio of UMN to LMN involvement, and rate of progression are each highly variable but independent of each other. (B) Early spread: As the disease process spreads neuroanatomically through UMN and LMN levels, clinical manifestations become complex due to differences (“incongruity”) between somatotopic anatomy and anatomic distances of the 2 levels. (C) Continued outward spread: For LMN, the ALS disease process continues to spread rostral-caudal (severity ipsilateral > contralateral) and must pass through the long thoracic region and thus appears to be mostly at one level. Degeneration may have preferential caudal spread (“directionality”) as discussed in the text. For UMN, however, the ALS disease process continues to spread medial-lateral and more quickly begins to appear as diffuse. (D) Advanced spread: Ultimately, degeneration appears to be diffuse and symmetric through temporal-spatial summation within and between UMN and LMN levels, the natural history of which has depended upon the features established at onset.

spread and the kinetics of underlying motor neuron degeneration. Progression rate has not been examined independently for UMN and LMN levels to determine if they are the same or different within each individual, but clinical observations suggest that they are similar. Variation in progression rates may reflect a dosage effect, the relative potency of a specific underlying pathogenic factor, or differences in disease-modifying genes—be they enhancers or suppressors of the pathogenic cascade.

### RECONCILING GENETIC WITH SPORADIC ALS

An important aspect of the pathogenic riddle is genetic disease, which currently accounts for as much as 18% of all ALS when “sporadic” cases tested for currently known genes are included. Since motor phenotypes of genetic and sporadic ALS are indistinguishable,<sup>43</sup> it is reasonable to expect molecular mechanisms are shared. Mendelian inheritance of ALS poses challenging questions: How can gene defects that are ubiquitously expressed be selectively

toxic? Why do gene defects remain dormant for decades before manifesting? And, we should add, how can ubiquitous gene defects produce focal pathology and contiguous spread? In relation to focality and spread, it seems reasonable that some combination of local and generalized abnormalities together produce motor neuron degeneration. The recent discovery that TDP-43 plays a major role in sporadic ALS and FTD,<sup>44,45</sup> that mutations in the *TARDBP* gene that encodes TDP-43 account for 3% of genetic ALS and up to 5% of sporadic disease,<sup>46</sup> and that pathologic depositions likely have a generalized topographic distribution all suggest that TDP-43’s role is fundamental and upstream in the pathogenic cascade similar to genetic mutations and is acted upon by the initiating molecular trigger and local mechanisms of propagation. This and the very recently identified *FUS/TLS* gene causing ALS6, both of which have biologically similar functions involving RNA processing, suggest RNA processing is a likely candidate for fundamental

abnormality in ALS.<sup>47</sup> That a generalized abnormality may be locally primed is a feature that is also consistent with environmental, toxic, infectious, or metabolic etiologies of ALS.

### FOCALITY AND SPREAD IN ALS HAVE IMPLICATIONS FOR RESEARCH DESIGN AND THERAPY APPROACH

Not only do focality and contiguity of ALS define key molecular mechanisms, but they also provide research opportunities. Because of them, death occurs when the orderly, topographic advance of the degenerative process affects neurons that control respiration and, consequently, death occurs before the degenerative process is universally complete<sup>48-50</sup> and while pathology is radially graded.<sup>25</sup> Hence, death is a point in time during the degenerative process, not the true end of the process,<sup>51</sup> a feature that contrasts with most other neurodegenerative diseases. Since nervous systems acquired rapidly can have high molecular quality,<sup>52</sup> cutting edge molecular and genomics approaches such as laser microdissection, RNA amplification, microarray, and computational biology can be directed to less affected regions to study early to moderately advanced stages of degeneration. The development of truly effective therapies will depend upon elucidation of specific molecular mechanisms and if focality is truly a feature of ALS, then therapies could be applied regionally at early stages to contain spread and efforts to spare the critical neurons that control respiration could be imagined. Only by clear understanding of the disease biology at the molecular level will such prospects be possible.

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