Deciphering Amyotrophic Lateral Sclerosis: What Phenotype, Neuropathology and Genetics Are Telling Us about Pathogenesis

Deciphering ALS: What Phenotype, Neuropathology and Genetics Are Telling Us about Pathogenesis

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Acronyms: ALS=amyotrophic lateral sclerosis; FALS=familial ALS; FTD=frontotemporal dementia; FTLD=frontotemporal lobar dementia; LMN=lower motor neuron; PLS=primary lateral sclerosis; PMA=progressive muscular atrophy; SALS=sporadic ALS; UMN =upper motor neuron

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is characterized phenotypically by progressive weakness and neuropathologically by loss of motor neurons. Phenotypically, there is marked heterogeneity. Typical ALS has mixed upper motor neuron (UMN) and lower motor neuron (LMN) involvement. Primary lateral sclerosis has predominant UMN involvement. Progressive muscular atrophy has predominant LMN involvement. Bulbar and limb ALS have predominant regional involvement. Frontotemporal dementia has significant cognitive and behavioral involvement. These phenotypes can be so distinctive that they would seem to have differing biology. But they cannot be distinguished, at least neuropathologically or genetically. In sporadic ALS (SALS), they all are characterized by ubiquitinated cytoplasmic inclusions of TDP-43. In familial ALS (FALS), where phenotypes are indistinguishable from SALS and similarly heterogeneous, each mutated gene has its own genetic and molecular signature. Putting this together, since the same phenotypes can have multiple causes including different gene mutations, there must be multiple molecular mechanisms causing ALS and ALS is a *syndrome*. But since multiple phenotypes can be caused by one single gene mutation, a single molecular mechanism can cause heterogeneity. What the mechanisms are remain unknown, but active *propagation* of the pathology neuroanatomically seems to be a principle component. Leading candidate mechanisms include RNA processing, cell-cell interactions between neurons and non-neuronal neighbors, focal seeding from a misfolded protein that has prion-like propagation, and fatal errors introduced during neurodevelopment of the motor system. If fundamental mechanisms can be identified and understood, ALS therapy could rationally target progression and stop disease—a goal that seems increasingly achievable.

INTRODUCTION

The chief characteristic that defines amyotrophic lateral sclerosis (ALS) is progressive weakness from neurodegeneration of the upper motor neuron (UMN) and lower motor neuron (LMN). *Clinically*, this is defined by a history establishing weakness over time and space, and by an examination showing signs of both UMN and LMN dysfunction in one or more body regions. *Neuropathologically*, ALS is defined by loss of motor neurons in brain, brainstem, and spinal cord and now increasingly by a sophisticated repertoire of neuropathological markers. Clinical phenotypes are determined by the *anatomic location* of neuropathology and during life, this anatomic pathology can be *imputed* clinically.

Heterogeneity of clinical phenotypes is characteristic—there are vastly different degrees of involvement of UMN and LMN, body regions that are affected, degrees of involvement of other systems especially cognition and behavior, and progression rates. While there are highly distinctive molecular neuropathological subtypes of ALS, in fact most of the known neuropathology seems more homogeneous than heterogeneous and does not clearly correlate with the various clinical phenotypes. This is the main mystery: is it one disease with shared fundamental biologic mechanisms or is it many diseases with different fundamental mechanisms and if so, how do they relate?

Genetics is giving us clues—clinical phenotype both masks and unmasks essential elements and there must be *both* single mechanisms and multiple mechanisms! Sporadic ALS (SALS), which is 90% of cases, and familial ALS (FALS), which is 10%, are indistinguishable from each other by phenotype. The fact that many different gene mutations have identical or at least highly similar clinical phenotypes tells us there must be multiple mechanisms that cause ALS and ALS is a syndrome. But the fact that one single genotype causes many different phenotypes tells us

that single mechanisms can lead to multiple phenotypes. Mechanisms both converge and diverge

CLINICAL PHENTOYPES

Clinical Phenotypes Based on *Level* of Involvement

"Typical" ALS (Tables 1 and 2): Weakness in classical ALS has simultaneous UMN and LMN characteristics. The weakness typically begins insidiously in discrete body regions and advances steadily over time and space. It begins in any of the three main body regions (face, arm and leg) although occasionally begins in the muscles affecting the trunk and/or respiration. This has been codified into the El Escorial criteria, which underscores that defining ALS syndromes is largely clinical and that the physical examination signs are critically important. The co-mixture of UMN and LMN clinical signs, indicating the degree to which the pathological burden is distributed between one level and the other, is normally distributed, with a possible skew to LMN predominance (1). At the extremes, when disease is predominantly UMN or predominantly LMN, there are special designations, highlighting the uniqueness and raising the question of whether or not these are fundamentally different biologically or the extremes of one continuum.

Primary lateral sclerosis (PLS) (Tables 1 and 2): PLS is the designation for a syndrome with solely UMN level phenotype and it remains unknown whether it is a discrete syndrome or a variant of ALS (2-7). In over half of PLS patients, symptoms begin insidiously in the legs and ascend smoothly and relatively symmetrically to arms and bulbar muscles. Others have a patchy progression, often with prominent bulbar symptoms. There is disagreement as the degree of LMN involvement especially as identified by EMG findings (8, 9). Patients with clinically pure PLS and no EMG changes four years after symptom onset have decades-long

survival (8, 10), whereas minor EMG or LMN findings predicted a poorer prognosis, consistent with typical ALS patients presenting with predominant UMN signs (11). Thus, the diagnosis of PLS should be made only after four years of disease duration (8). PLS may stabilize after a few years of progression (12), although similar stabilization may occur in UMN-dominant ALS (UMN-D ALS) (11, 13). Frontotemporal dementia (FTD), cognitive impairment and altered behavior occur in PLS comparable to ALS (14). Ultimately, PLS is a clinical diagnosis that relies upon exclusion of other known causes of progressive spasticity, such as sporadic presentations of hereditary spastic paraparesis (15).

Progressive muscular atrophy (PMA) (Tables 1 and 2): PMA is the designation for syndromes with predominantly LMN phenotype. Onset can be in any body region and compared to ALS, PMA patients are more likely to be men and have a higher age of onset. Approximately 30% of PMA patients develop UMN signs within 18 months (16, 17). A subset of patients that is characterized by segmental involvement for more than 4 years duration have slow progression and prolonged survival, though transition to ALS can occur even in this group (18, 19). Patients with PMA demonstrate the same frontotemporal pattern of cognitive involvement as is seen in typical ALS and thus the degree of UMN involvement does not correlate with cognitive involvement (20). The UMN imaging data is not straightforward. Depending on technique, changes may not be seen (21), may predate clinical changes (22), or may show extra-motor white matter involvement (23). By MR spectroscopy, one study showed changes in 63% of PMA patients (24) and another study showed only modest, nonsignificant changes (25). Neurophysiological studies of central motor conduction using transcranial magnetic stimulation show abnormalities in 50 - 63% of patients with clinical PMA (24, 26).

Clinical Phenotypes Based on *Body Region* of Involvement

Bulbar and pseudobulbar palsy (Tables 1 and 2): While the designations PLS and PMA are based on the *level* of the underlying pathology, another set of designations is based on the *body region* first affected at the outbreak of disease. When ALS begins by affecting the muscles of speech, mastication and swallowing, it is designated bulbar-onset ALS. The designation *bulbar* has traditionally signified predominantly LMN involvement and the designation *pseudobulbar* has traditionally signified predominantly UMN involvement, but often *bulbar* is used as parlance for both. EMG positive (meaning LMN is involved) and EMG negative (meaning only UMN involvement) have similar progression. Interestingly, there is a female predominance in bulbar palsy, compared with other limb regional forms where there is male predominance. The bulbar system is more complicated than the arms and legs and thus is more than just a fifth limb. Bulbar onset is more highly associated with affect and cognition and often has the added feature of altered and exaggerated emotional expression that is called emotional incontinence and delineation has permitted a clearer understanding of the natural history (27). Bulbar symptoms are often directly correlated with depression. Neurophysiological studies have identified neural networks underlying corticobulbar control of swallowing that are especially affected during repetitive movements (28). Functional MRI studies of the course of bulbar and limb- onset ALS are providing insights into the interrelationship between brainstem derived and spinal cord derived neural networks (29). A treatment based on dextromethorphan has an attenuating effect on pseudobulbar affect.

Limb regional variants including flail leg, flail arm, polyneuritic pattern, and hemiplegia (Mills's variant) (Tables 1 and 2): When ALS begins by affecting muscles of the limbs, as it does two thirds of the time, it is referred to as limb-onset, or typical, ALS as discussed above. But within this group, a few variant phenotypes have stood out and been given special designations, with a view that these variants may have variant biology. Typically these variants are predominantly LMN syndromes and tend to be very slowly progressive.

Upper Extremity Regional Variant: This is a regional variant consisting of weakness exclusively confined initially to the upper extremities and cases have also been described under the names of “hanging arm syndrome”, “neurogenic man-in-the-barrel”, “flail arm syndrome”, “brachial amyotrophic diplegia”, and the “Vulpian-Bernhart syndrome”. These patients have bilateral upper extremity weakness and atrophy that affects predominantly the proximal arms and shoulder girdle (19, 30). The average age of onset does not differ from that of ALS, but compared with ALS, this syndrome is significantly more common in men. Average survival is approximately 5 years, however, the definitions used for these patients has been slightly different. Some patients presenting with this phenotype can go on to develop a typical ALS course. Katz et al used an 18-month time of weakness confined to the arms, and no UMN signs; Wijeskera used 12 months and patients could have UMN signs. In the original series of Katz, after a mean follow-up of 5.5 years, weakness remained restricted to the upper extremities in 7 out of 19 patients.

Lower Extremity Regional Variant: This LMN variant confined to the legs is known as the pseudopolyneuritic variant of ALS, the Marie-Patrikios form, flail leg syndrome, the peroneal form of ALS, and leg amyotrophic diplegia (19, 31, 32). It is rare (about 3-3.5% of all motor neuron disease cases), predominantly male, predominantly LMN, and relatively slowly progressive with mean survival ranging from 76 to 96 months.

Mill’s Variant (Hemiplegic ALS): This is a disputed rare variant phenotype characterized by a progressive hemiplegic pattern of motor deficit that ascends from the leg or descends from the arm. This could represent a variant of PLS. The scarce literature that exists suggests that it is simply a descriptive clinical term. A PET study in one such patient demonstrated a striking lateralization of microglial activation in the hemisphere contralateral to the hemiplegia (33).

Clinical Phenotypes with Involvement of Non-Motor Regions

Frontotemporal dementia (Tables 1 and 2): The overlap of FTD and ALS has been well documented in FTD patients with co-morbid motor neuron degeneration and in ALS patients with frontotemporal dysfunction (34-37). Up to 15% of FTD patients and 30% of ALS patients experience the overlap syndrome. The syndrome may be difficult to identify since ALS patients' behavioral or cognitive abnormalities may be subtle and since patients often present either to a neuromuscular clinic or a memory disorders center. New designations called behaviorally impaired and cognitively impaired ALS were created to reflect uncertainty as to whether or not they may have different underlying biologies (38). Key tests that are useful looking for cognitive behavioral impairment and excluding depression are beginning to emerge (39). Survival is impacted for both disorders in the co-morbid condition, making identification of this syndrome critical. There is a survival difference of more than a year between patients with comorbid disease versus ALS alone (40).

Other system involvement: In addition to dementia, other systems can be involved in what otherwise seems to be typical ALS. These include the extrapyramidal motor systems (41-47), supranuclear gaze systems (48-51) and the autonomic nervous system (52, 53). Increasingly, defects in energy metabolism including weight loss, hypermetabolism and hyperlipidemia have been identified and implicate either that other CNS regions such as hypothalamus are involved or that ALS is part of a systemic disease or both (reviewed in (54)). Such "atypical" involvement is sometimes referred to as "ALS-plus syndromes" but there is ample clinical, neuropathologic and neuroimaging evidence to suggest that these should be considered to be part of the neuropathologic spectrum of ALS/MND (55).

MOLECULAR NEUROPATHOLOGY

Overview neuropathological heterogeneity

Clinical phenotypes are based on the anatomy of the neuropathology *imputed* by clinical localization early in the disease course as summarized in Table 2—actual neuropathology is studied after changes have summated over time through the course of the disease. Surprisingly little data is available on their correlations. Neuropathologically, ALS is defined as the loss of UMNs (commonly thought of as Betz cells in layer V of area 4 of Brodmann) and LMNs (commonly thought of as alpha motor neurons in the motor nuclei of the brainstem and Rexed Lamina IX of the anterior horns in the spinal columns). Wallerian/axonal degeneration in the projecting pathways from the UMN is seen in the corpus collosum, centrum semiovale, internal capsule, cerebral peduncle, basis pontis, medullary pyramids and lateral columns ("lateral sclerosis") and similarly degeneration in the projections from the LMN is seen in the anterior roots and peripheral nerve, leading to muscle denervation ("amyotrophy"). In addition, there is astrogliosis, spongiosis, and microglial activation, which are thought to represent mainly secondary reactive changes, at least neuropathologically, although basic research is supporting a more primary role for non-neuronal cells. The neuropathology that is reported on PMA and PLS suggests these ALS clinical phenotypes share a similar neuropathology and their differences are more likely based on the anatomical distribution of the pathological burden than on biological differences selecting one level or region over another or on the molecular characteristics. 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 (56). 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 (57). PLS neuropathology shows changes in the LMN and these changes are of the same molecular pattern as is seen in typical disease (58, 59).

Distinctive molecular neuropathological types (Table 3)

In 1988, Leigh et al and Lowe et al, independently identified depositions of ubiquitin in the cytoplasm of ALS motor neurons using immunohistochemistry (60, 61). The morphologies of the deposits were either skein-like or dense and round. Ubiquitin is a housekeeping protein involved in protein homeostasis and the finding suggested an unknown pathological protein was being tagged for removal by the cell. Similar changes of ubiquitinated aggregates were soon identified in about 50% of brains from FTD patients (discussed below). In 2006, the identity of the ubiquitinated protein in both diseases was found to be TDP-43, a nuclear protein involved in DNA and RNA processing and that in these diseases translocated to the cytoplasm, became cleaved, hyperphosphorylated and insoluble (62, 63). Our current molecular neuropathological classification is likely to be continually modified: it now appears that the other proteins beside TDP-43 may be ubiquitinated; newer markers are being identified; and abnormal TDP-43 may be seen in other neurodegenerations. But for now, overall, essentially all *sporadic* ALS and nearly all familial ALS except SOD1- and FUS- associated ALS, regardless of clinical phenotype including PLS and PMA, seems to have as its hallmark neuropathological pattern deposition of ubiquitinated TDP-43 in the cytoplasm of CNS cells, leading to a belief that ALS is a TDP-43 proteinopathy. Heat maps of the distribution of TDP-43 pathology show that abnormalities are widely present in the brain, not just in motor regions (64).

GENETICS

Clinical Phenotypes Based on Genetics

Five to 10% of ALS is genetically transmitted mainly by way of dominant gene mutations and these numbers increase to as high as 15-20% when known genes are tested in patients who were

thought to have sporadic disease. Approximately 60-70% of the genes have now been identified, the main ones being *SOD1*, *TARDBP*, *FUS* and *C9ORF72* (reviewed in (65)). Clinical phenotype heterogeneity of FALS is as characteristic and vast as SALS, and no clinical features easily distinguish one from another. Remarkably, this clinical phenotype heterogeneity is even seen in the same mutation in the same gene in the same kinship, implying phenotype is likely determined by factors other than the molecular cascade it triggers. But some trends exist. Mutations in *SOD1* and *FUS* tend to cause predominantly LMN syndromes. Mutations in *TARDBP* tend to begin in the upper extremity and to progress slower than average (66). Mutations in *SOD1*, *TARDBP* and *FUS* cause mostly motor syndromes and are only rarely associated FTD. Mutations in *FUS* cause a juvenile as well as adult motor neuron disease syndrome. Mutations in *C9ORF72* are as likely to cause FTD as ALS, often with psychosis. The 'A4V' mutation in *SOD1* ALS is rapid while the 'D90A' mutation, unusual in that it is recessive, is slow and indolent.

Genetically defined ALS neuropathology (Table 3)

Whereas gene mutations do not directly correlate with clinical phenotype, they do correlate with molecular neuropathology, which seem to be distinctive among the various genes. (reviewed in (67). The *first* and main molecular neuropathological subtype is TDP-43 proteinopathy as defined above. This applies to all sporadic ALS and most non-*SOD1* familial ALS. Ubiquitin and TDP-43 positive skeins define it and dense round inclusions deposited in the cytoplasm in the spinal motor neurons and the cortex, where they are primarily localized in motor areas (68). The inclusions are also seen glial cells.

The *second* and newest defined neuropathological subtype of ALS is a variation on TDP-43 proteinopathy related to expanded hexanucleotide repeats in *c9orf72*, which represents 33-40% of FALS and up to 7% of SALS. *C9ORF72*-associated cases have all the hallmark features of

TDP-43 proteinopathy, and, in addition, there are also abundant deposits of p62 and other markers in the cytoplasm and nucleus of neurons in the cerebellum, basal ganglia and hippocampus, features which are not robust in non-*C9ORF72*-associated cases (69, 70). p62 is a protein involved in both the proteosomal pathway and in autophagy, and this has relevance to the growing interest in these pathways in neurodegeneration.

The *third* main molecular neuropathological signature of ALS applies to mutations in *SOD1*, which represents up to 20% of FALS and 1-2% of SALS. Most of the *SOD1*-associated neuropathology was reported prior to 2006 and TDP-43 and hexanucleotide repeat expanded *C9ORF72* were identified and sorely needs to be updated (discussed by (71)). But in general, it is characterized by deposition in the cytoplasm of sometimes large amorphous conglomerates of ubiquitinated SOD1 protein that are negative for TDP-43 (68). There seems to be a greater burden on the LMN than UMN (72) and the degree of axon loss seem to be greater than neuronal loss, leading to the concept of it being a distal axonopathy (73). Misfolded SOD1 is present in *SOD1*-associated FALS but whether or not this is significant in SALS (74, 75) is disputed by emerging evidence that suggests if it is present at all, it is not prominent (76-78).

The *fourth* main molecular neuropathological signature of ALS is FUS proteinopathy, which represents up to 3% of FALS and less than 1% of SALS. This neuropathological subtype is characterized by basophilic inclusions in the cytoplasm of neurons of the motor cortex and of spinal anterior horns, and by FUS-positive, TDP-43-negative immunoreactive inclusions in the cytoplasm of neurons and glia in the motor cortex, spinal anterior horns and various non-motor regions (79, 80). There appear to be different signatures in juvenile and adult forms of disease (80, 81). One recent report indicated FUS proteinopathy might be prominent in TDP-43-proteinopathies if optimal technical protocols are used for detection (82), but so far this has not been verified by others.

WHAT DO CLINICAL HETEROGENEITY AND PATHOLOGICAL HOMOGENEITY INDICATE ABOUT UNDERLYING MECHANISMS?

Hypotheses of propagation

How phenotype, neuropathology and genetics relate to each other is not understood, but *propagation of pathology* has been identified as a principle component of pathobiology that could provide unified explanation. One hypothesis posits *neuroanatomic propagation* (83). ALS usually begins in discrete body regions and for these regions, the degree to which UMNs and LMNs are affected (the *distribution* of disease burden) is variably distributed (1). Once triggered, disease propagates to proximate neuroanatomical regions independently at the two levels and progressive motor neuron loss summates and then saturates neuropathologically (84). The rate of disease progression reflects both the *kinetics* of propagation and the *distribution* of the disease burden between UMN and LMN. In this light, PLS and PMA differ primarily in distribution of the pathologic burden between UMN and LMN levels, limb variants differ by neuroanatomic location of onset, and FTD and ALS differ in the cerebral distribution of pathology. A dramatic example is repeat-expanded *C9ORF72*-associated ALS/FTD, where a single disease mechanism leads to either ALS or FTD phenotype, each with their own heterogeneity.

Different from neuroanatomic propagation is a hypothesis of *propagation within neuronal networks*. According to this hypothesis, the vast functional as well as structural networks in the CNS create a connectome (85). Neuronal networks may have selective vulnerabilities through natural anatomical patterns that underlie different neurodegenerative syndromes (86), possibly

through preferential spread (87). In support of this, advanced MRI data demonstrating ALS-specific neurodegeneration within motor and extramotor networks is emerging (88-90).

While propagation patterns have now been defined by many groups (91-95), other contributions to pathobiology are also emerging. One recent study identified that up to 14% of second regions involved in disease progression were not contiguous (95). Bifocal or multifocal onset has been proposed (96). Two recent studies using different approaches, one traditional groupings and one unbiased cluster analysis, identified a variety of demographic factors that are significant determinants of phenotype, (93, 97). Genetic syndromes, which are often focal in onset, also have other important biological determinants of phenotype based on factors other than propagation such as the tendencies for mutations in SOD1 and FUS tend to cause predominantly LMN syndromes, mutations in SOD1 and TARDBP to cause mostly ALS rather than FTD, and mutations in C9ORF72 to cause as much FTD as ALS.

Parallels to FTD

ALS and FTD overlap in many characteristics and they are increasingly viewed as part of a pathobiological spectrum. FTD has three main clinical phenotypes: primary progressive aphasia, semantic dementia and behavioral variant. As with ALS clinical phenotypes, here too the prime determinant is the focal neuroanatomic site of onset—left or right frontal or temporal regions. The neuropathology of FTD, referred to as frontotemporal lobar dementia (FTLD), has three main subtypes (Table 3): FTLD-U or TDP-43 (about 50%), FTLD-FUS (about 3-5%), and FTLD-tau (about 40-50%). FTLD-U or TDP-43 and FTLD-FUS overlap significantly with ALS, but FTLD-tau does not, except in one recent report (98). A main genetic overlap between ALS and FTD is with hexanucleotide repeat expanded C9ORF72 families, and lesser degrees of overlap are seen with the other ALS genetic mutations (reviewed in (67). Extensive studies over the last several years have sought correlations between FTD clinical phenotypes with FTLD

neuropathological and genetic subtypes, and while trends have been identified, predictors and correlations are unclear and complex. The fact that FTD phenotypes can be as defined by the focal neuroanatomic site of onset as they can by any neuropathological or genetic feature suggests that FTD, like ALS, may be viewed as a focally beginning and propagating disease and that, in turn, the pathobiology of propagation is a principle a component of its biology.

PATHOGENESIS

Prime observations: It is reasonable to divide pathogenic mechanisms into three separate components: *triggers, progression (or propagation),* and *neuronal death*. The separable importance of disease *trigger* is highlighted by the observation that onset is highly variable in site and in distribution between UMN and LMN, in penetrance, and in age of onset. The separable importance of *disease progression* is highlighted by the highly variable patterns of progression and the progression rates, which suggest variable kinetics of propagation both in space and time. The separable importance of *cell death* is highlighted by the observation that select motor neuron degeneration is the ultimate result neuropathologically and that this summates over time and space. Many themes about molecular mechanisms of neurodegeneration have emerged over the past two decades and some of these are highlighted next.

Motor neuron resistance and vulnerability in ALS: In ALS, the most vulnerable UMNs are layer V projection neurons in the primary motor cortex, and spinal motor neurons of the ventral horn. The most vulnerable regions and neurons in FTD are less clearly defined, but anterior cingulate and frontoinsular regions show early involvement, and these regions contain unique layer V projection neurons (von Economo neurons and fork cells). The coincidence of ALS and FTD in some patients raises the possibility that a shared cellular or molecular feature is present in

cortical motor neurons and subspecialized layer V neurons of other cortical regions, which defines the sensitivity to degeneration in ALS/FTD. The prominent layer V degeneration in TDP-43 transgenic models suggests the determinants of this shared vulnerability of layer V neurons may be present in rodents and be accessible to study. Improving our understanding of whether subspecialized layer V cells really are selectively vulnerable in ALS and FTD, and why, could provide a unique angle for understanding pathogenesis of these diseases.

Among different LMN populations, the motor neurons subserving eye movements and pelvic sphincters are highly resistant compared with typical spinal motor neurons. Although often considered as a group, spinal motor neurons are highly diverse in terms of their morphology, connectivity, and functional properties and differ significantly in their response to disease. Recent studies of motor neuron diversity have clarified developmental mechanisms and provided novel insights into their neurodegeneration. Motor neurons of different classes and subtypes—fast/slow, alpha/gamma—are grouped together into motor pools, each of which innervates a single skeletal muscle. Distinct mechanisms regulate their development. In multiple contexts including ALS, spinal muscular atrophy and aging, fast-fatigable motor units degenerate early compared to motor neurons innervating slow muscles. Mechanisms for this could also relate to those conferring resistance to of those subserving eye movement and pelvic sphincter control. If we could understand why populations and subpopulations of motor neurons are resistant or vulnerable, we would have a strong rationale approach for intervention. One approach is through functional genomics using laser capturing and new genomic technologies. Extrinsic and intrinsic mechanisms that confer resistance represent promising therapeutic targets in these currently incurable diseases.

ALS as a systemic disease: As previously stated, there is a growing body of knowledge about the systemic changes that are occurring in ALS. These include ultrastructural abnormalities in hepatic cells, skin cells, muscle mitochondria, systemic glutamate metabolism, inflammatory cytokine production, immunological changes, glucose metabolism, and lipid metabolism. Skeletal muscle is the single largest organ by mass, constituting 40-45% of the entire body mass and is the end-organ of the motor neurons. Skeletal muscles generate target-derived neurotrophic factors that can substantially affect motor neuron survival. Part of the hypermetabolism that is becoming defined in ALS patients may be due to due to abnormal mitochondrial energy production in skeletal muscle(99), generating a large amount of radical oxygen species (100) that could interact with those from inside the CNS (101). Lipid peroxidase products are highly biologically active, causing cellular damage via apoptosis or nucleophilic action and these could be connected to ALS by way of APOE isoforms and/or paraoxonase I (PON1) or other pathways.

The cellular neighborhood matters—Non-autonomous cell death: It is now clear that ALS associated with *SOD1* mutations is non-cell autonomous, that is damage of the population of affected neurons depends upon complex interactions between them and their surrounding cells (102, 103). From analysis of mice that are mixtures of mutant-expressing SOD1 and normal cells, gene inactivation in selected cell types, and cell grafting to replace mutant expressing cells with normal ones, it appears mutant damage within motor neurons determines the timing of disease onset mutant damage within astrocytes and microglia drives disease progression. Thus, the cellular neighborhood matters. The exact roles of the different cell types are complex. Astocytes expressing ALS-related *SOD1* mutations, can kill neighboring spinal motor neurons and are crucial to drive disease progression. This mechanism is unknown but the preponderance of evidence, from sporadic and familial ALS as well as rodent models, suggest a common loss of function of glutamate handling, through decreased expression and function of

glutamate transporters(104-106), which is neurotoxic. Another could be mediated by a soluble toxic factor(s) that is protein in nature, thermo-labile, and negatively charged, but no *in vivo* evidence has emerged for this gain-of-toxicity mechanism. The identity of this toxic factor(s), the molecular pathways engaged, and protective small molecules have not yet identified exactly how this occurs. Microglia, the resident innate immune sentinels of the CNS, become activated, and evidence from both *in vivo* and *in vitro* models suggests that they can be either neuroprotective or cytotoxic, probably through the release of neurotrophic factors and cytokines (107-109). Activated microglia may switch from anti-inflammatory and neuroprotective to proinflammatory and neurotoxic, and a greater understanding of the numerous pathways involved could provide opportunities for novel therapeutic intervention. Oligodendroglia in the grey matter have recently been found to have a significant role in ALS. They are derived from NG2 cells, which are adult stem cells located throughout the neural axis. Oligodendrocytes provide trophic and possibly metabolic support to neurons and axons. They are massively proliferating in ALS, both mouse models and human disease, either because of some unknown signal or oligodendrocyte injury. Their exact role in ALS neurodegeneration and how this discovery may impact therapy remains to be determined.

RNA processing and RNA toxicity: Views on pathogenesis are undergoing a sea-change from the recognition of the importance of the two RNA/DNA-binding proteins TDP-43 and FUS. Both are widely expressed, predominantly nuclear, have similar domains and prion-like properties, and have ALS mutations localized in the C-terminal region. They are both structurally close to the family of heterogeneous ribonucleoproteins (hnRNPs) and have been involved in multiple levels of RNA metabolism including transcription, RNA splicing, RNA transport, translation and microRNA processing (reviewed in (110-112). Importantly, splicing alterations (113, 114) and mRNA-editing errors (115-117) have been reported in sporadic ALS patients, albeit a role of TDP-43 or FUS in these modifications has not been defined. The emerging TDP-43 and FUS stories have led to the proposal that defects in RNA processing play a central role in

neurodegeneration and this was further underscored by the recent recognition of intermediate length polyglutamine expansions in ataxin-2, another RNA binding protein, as a risk factor for ALS (118). At present, it is unresolved as to whether neurodegeneration is due to a loss of TDP-43 or FUS function, a gain of toxicity or a combination of the two. The nuclear clearance of TDP-43, and to less extent FUS, in neurons containing cytoplasmic aggregates, is consistent with pathogenesis driven, at least in part, by a loss of TDP-43 or FUS nuclear function. An alternative (not mutually exclusive) hypothesis, however, is that the accumulated proteins acquire a toxic function in the cytoplasm of affected neurons. This acquired toxic function may also rely on the RNA-binding properties of these proteins, as suggested by recent works in yeast, fruit fly and chick showing that the toxicity of TDP-43 aggregates is abolished when the RNA-binding property of the protein was removed (118, 119).

In October, 2011, an expanded hexanucleotide repeat in *C9ORF72* was identified in chromosome 9-linked ALS, FTD and their overlap, thus identifying the single most common genetic mechanism in ALS/FTD. This identification has three immediate implications. First, the same genetic defect can cause either ALS or FTD phenotype, thus re-enforcing that clinical phenotype does not directly reflect underlying molecular mechanism. Second, ALS and FTD now joins the group of expansion repeat disorders, a group of >22 inherited neurodegenerative diseases characterized by expanded nucleotide repeat sequences (microsatellites) in the genome. Third, two mechanisms seem most reasonable: gain-of-function due to production of toxic RNA; or loss of gene function, although no known functions of the *C9ORF72* protein are currently known. A third possibility, that the protein acquires a toxic function, seems unlikely since the expanded repeat is intronic.

Prion-like propagation: ALS and the linkage of ALS to FTLD could be explained on the basis of disease proteins such as SOD1 and TDP-43 propagating pathologically from cell to cell. This

theme is emerging in a variety of different neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, as well as FTD and ALS, as it emerges that a number of proteins including alpha-synuclein, tau, abeta and mutant SOD1 may propagate within the central nervous system. Transmission or propagation is not the same as infectivity and the terms being used for these properties are “prion-like” or “prionoids” (Aguzzi, 2009), to distinguish them from bona fide prions, which are infectious. To date, there is no evidence that any other neurodegenerative disease besides prion diseases can be acquired by infection in humans (reviewed in (120)). Nevertheless, disease progression within the same individual, from a focal site of initiating damage throughout the central nervous system has been described for many neurodegenerative diseases, including ALS. While the molecular basis of these observations is not well understood, the propagation of pathological conformation of disease-related proteins (pathological templating) could underlie this phenomenon. Indeed, misfolded SOD1 and TDP-43 were recently shown to induce a pathologic conformation on their natively folded counterparts when introduced on cells in culture (reviewed in (120)). This behavior is reminiscent of the pathologic prion protein and has now been demonstrated for several proteins that misfold and accumulate in neurodegeneration, including SOD1 and TDP-43 as well as A-beta, tau, and alpha synuclein (121-123). Preformed fibrils generated from recombinant alpha-synuclein, for example, when dripped onto primary cultures of wild-type neurons, will induce alpha-synuclein Lewy neurite pathology in processes, and this gets transported retrogradely back to the cell body where Lewy bodies are formed (124). Physical application to the cell bodies results in its transportation in the opposite direction, and there appears to be transmission throughout other parts of the brain. Not every neuron will be affected in the neuroanatomical pathways that connect one part of the brain to the other, but many are. And glial cells also can be induced to form alpha-synuclein pathology, at least in the transgenic mice.

"Molecular logic" of corticospinal motor neuron development, degeneration, and subcerebral projections: Interesting suggestions were made that common molecular origin during the development of corticospinal motor neurons (CSMN) and related subsets of cortical non-motor, cognitive association "subcerebral projection neurons" might explain at least some of the cognitive aspects of ALS (125). ALS might result, at least in part, from neuronal subtype-specific vulnerability due to errors introduced during neurodevelopment. Complex molecular controls regulate specification, differentiation, connectivity, and survival to create enormous complexity of CNS neuronal subtypes and their connections. Results over the past several years identify that the development and maintenance of corticospinal motor neurons and other neocortical projection neuron populations are controlled by a combinatorial set of complexly interacting developmental molecular regulators, largely transcription factors and co-regulators (126). These control key developmental processes including progenitor parcellation, subtype-specific differentiation, area identity, and axonal outgrowth (127). Loss-of-function and gain-of-function analyses for identified genes and molecules reveal a nested "molecular logic" of progenitor-stage and post-mitotic molecular controls, many allele-specific and matching human disease distributions that exert either specific CSMN control or shared control over CSMN and related non-motor sub-cerebral projection neurons. The molecular-genetic controls occur in multiple steps and are parcellated in space, separated in distinct neuron subtypes in the same spatial position, and orchestrated over time via cross repression and acquisition of sequential stages of development, among other mechanisms. Most relevant to ALS and related UMN disorders and to disorders with non-motor, cognitive or sensory involvement, it now appears that a specific subtype of progenitors generates the entire set of CSMN, related subcerebrals, and corticothalamic projection neurons– all "corticofugal projection neurons". Then, CSMN and all other subcerebrals share a distinct set of controls that are different from corticothalamics. Thus, CSMN and non-motor, cognitive and sensory subcerebrals are built on a "common chassis", and common molecular abnormalities can predispose this broader population, or many narrower

and more specific populations, to selective disease vulnerability, e.g. UMN disease with more or less non-motor involvement. Many developmental genes have now been identified as being associated with classical ALS. Thus, during initial development, errors might be introduced that lead to selective vulnerability and later degeneration.

CONCLUDING REMARKS

The holy grail of ALS is rationally designed therapy that effectively stops ALS neurogeneration in its advance. The fact that different gene mutations cause identical clinical phenotypes means that multiple mechanisms exist and ALS is a syndrome. But the fact that one single gene mutation causes many different ALS phenotypes means that there must be single common mechanisms and propagation of pathology is a principle component. With the transforming understanding of clinical, neuropathological, and molecular-genetic aspects of ALS over the last five years, this quest for rational fundamental therapy has become a realistic hope.

TABLE 1. ALS CLASSIFICATIONS

Phenotype	Molecular Pathology	Genetics
Typical ALS Bulbar/pseudobulbar ALS Limb onset variants: • Typical limb onset • Flail arm or bibrachial ALS • Flail leg • Polyneuritic variant • Hemiplegic ALS (Mill's variant) ALS with associated FTD or impairment of higher cortical function Primary lateral sclerosis Progressive muscular atrophy	TDP-43 proteinopathy (ubiquitinated pathology) • Without repeat expanded C9ORF72 • With repeat expanded C9ORF72 SOD1 proteinopathy FUS proteinopathy (basophilic inclusion body disease (BIBD))	Sporadic ALS Familial ALS, incl.: • ANG • C9ORF72 • FIG4 • FUS • OPTN • SOD1 • TARDBP • UBQLN • VAPB

TABLE 2. PHENOTYPE CLASSIFICATION BASED ON *CLINICALLY IMPUTED* ANATOMY OF NEUROPATHOLOGY

		CNS Anatomical Region Involved*			Innervated Peripheral Body Region Involved*		
		UMN	LMN	Fronto-temporal regions	Bulbar muscles	Limb muscles	Higher cortical function & behavior
Based on *Level* of Involvement	ALS	++	++	+/-	++	++	+/-
	PLS	++++	+/-	+/-	++	++	+/-
	PMA	+/-	++++	+/-	+/-	++++	+/-
Based on *Body Region* of Involvement	Bulbar ALS	+/-	++++	+/-	++++	+/-	+/-
	Pseudobulbar ALS	++++	+/-	+/-	++++	+/-	+/-
	Limb ALS & variants	+/-	++++	+/-	+/-	++++	+/-
Associated cognitive	FTD or behavioral/cog	+/-	+/-	++++	+/-	+/-	++++

* +/- possible but not typical; ++ typical and to variable degree; ++++ primary feature

changes	nitive impairment						

TABLE 3. ALS PROTEINOPATHIES: MAIN MOLECULAR NEUROPATHOLOGICAL FEATURES

Proteinopathy	Phenotype	Gene	Main Molecular Features†				
			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

† NCI = nuclear cytoplasmic inclusions; GCI = glial cytoplasmic inclusions

• **SOD1 pathology‡**	• ALS, usually LMN predom-inant 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 protein-**	• 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

‡ No primary FTD phenotypes have been defined by SOD1 pathology.

opathies • **Ubiquitinated pathology**							
• **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 is present but separable from p62 & UBQLN	• Degeneration & sclerosis
• **Tau pathology (including FTLD-Tau with Pick bodies)[§]; Tauopathies**	• FTD • Progressive supranuclear palsy • Corticobasal	• MAPT	• Signature tau+, ubiquitin-TDP43- NCIs and GCIs	• Few reports, presumptively negative (see §)	• Signature tau+, ubiquitin-, TDP43-NCIs and GCIs	• Pick bodies = 3R tau+ globular or spherical NCIs in the granule cells of dentate gyrus;	• Presumptively negative

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

	syndrome • Multiple system atrophy						

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