Article:
Turner, M.R., Bowser, R., Bruijn, L. et al. (2013) Mechanisms, models and biomarkers in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 14 (Sup1). pp. 19-32. ISSN: 2167-8421

https://doi.org/10.3109/21678421.2013.778554

Published in final edited form as:
Amyotroph Lateral Scler Frontotemporal Degener. 2013 May ; 14(0 1): 19–32. doi: 10.3109/21678421.2013.778554.

Mechanisms, models and biomarkers in amyotrophic lateral sclerosis

Martin R. Turner[1], **Robert Bowser**[2], **Lucie Bruijn**[3], **Luc Dupuis**[4,5], **Albert Ludolph**[5], **Michael Mcgrath**[6], **Giovanni Manfredi**[7], **Nicholas Maragakis**[8], **Robert G. Miller**[9], **Seth L. Pullman**[10], **Seward B. Rutkove**[11], **Pamela J. Shaw**[12], **Jeremy Shefner**[13], and **Kenneth H. Fischbeck**[14]

[1]Nuffield Department of Clinical Neurosciences, University of Oxford, UK [2]Division of Neurology, Barrow Neurological Institute, Phoenix, Arizona [3]The ALS Association, National Office, Washington DC, USA [4]INSERM U692 & Université de Strasbourg, Strasbourg, France [5]Department of Neurology, Ulm University, Ulm, Germany [6]UCSF, San Francisco, CA [7]Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, NY [8]Johns Hopkins University, Department of Neurology, Baltimore, Maryland [9]Forbes Norris ALS Research Center, California Pacific Medical Center, San Francisco, California [10]Columbia University, New York, NY [11]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA [12]Department of Neuroscience, Sheffield Institute for Translational Neuroscience, University of Sheffield, UK [13]Department of Neurology, SUNY Upstate Medical University, Syracuse, NY [14]Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

Abstract

The last 30 years have seen a major advance in the understanding of the clinical and pathological heterogeneity of amyotrophic lateral sclerosis (ALS), and its overlap with frontotemporal dementia. Multiple, seemingly disparate biochemical pathways converge on a common clinical syndrome characterized by progressive loss of upper and lower motor neurons. Pathogenic themes in ALS include excitotoxicity, oxidative stress, mitochondrial dysfunction, neuroinflammation, altered energy metabolism, and most recently RNA mis-processing. The transgenic rodent, overexpressing mutant superoxide dismutase-1, is now only one of several models of ALS pathogenesis. The nematode, fruit fly and zebrafish all offer fresh insight, and the development of induced pluripotent stem cell-derived motor neurons holds promise for the screening of candidate therapeutics. The lack of useful biomarkers in ALS contributes to diagnostic delay, and the inability to stratify patients by prognosis may be an important factor in the failure of therapeutic trials. Biomarkers sensitive to disease activity might lessen reliance on clinical measures and survival as trial endpoints and reduce study length. Emerging proteomic markers of neuronal loss and glial activity in cerebrospinal fluid, a cortical signature derived from advanced structural and

Correspondence: M. R. Turner, Clinical Neurosciences, West Wing Level 3, John Radcliffe Hospital, Oxford OX3 9DU, UK. martin.turner@ndcn.ox.ac.uk.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

functional MRI, and the development of more sensitive measurements of lower motor neuron physiology are leading a new phase of biomarker-driven therapeutic discovery.

Keywords

ALS; biomarkers; pathogenesis; neuroimaging; neurophysiology

Introduction

There is an intense need to establish biomarkers sensitive to diagnosis, prognostic stratification and disease activity of ALS (Table I). The diagnosis of amyotrophic lateral sclerosis (ALS) currently depends upon the opinion of an experienced neurologist, and the exclusion of potential mimic disorders. In the context of progressive weakness, where there are typical upper (UMN) and lower motor neuron (LMN) signs within the same body region, there is little diagnostic doubt and few tangible mimics. However, such obviously mixed clinical signs may not be apparent at presentation (or indeed ever) for a large proportion of patients, who are nonetheless considered to form part of the wider syndrome of ALS (1). This syndrome is currently unified by the post mortem finding of cytoplasmic inclusions of the protein TDP-43, with the notable exception of those with mutant *SOD1* gene-associated ALS (approximately 1% of all cases) in whom the disease is nonetheless clinically indistinguishable (2). The failure of multiple drugs tried for the treatment of ALS may in part be a function of their application relatively late in the course of the disease, given the average diagnostic delay of one year (3). It is not yet known when the initial pathological changes of ALS begin, but it seems likely that the clinical manifestations occur significantly downstream of what may be rapidly irreversible primary events at the cellular level. Earlier diagnosis would permit introduction of therapies nearer to these initiating events.

ALS is usually an apparently sporadic disorder and the insidious clinical onset involves symptoms common to more benign disorders, which means that a significant proportion of the diagnostic latency may remain inaccessible. At the time of diagnosis, however, there is additional value in identifying biomarkers sensitive to the recognized variation in progression rate. Although the median survival from symptom onset in ALS is less than three years, the second half of the survival curve is longer, and beyond a decade for at least 5% of patients. The presence of relatively ‘pure’ UMN or LMN clinical signs is associated with longer survival (4, 5), with the rare UMN-only variant primary lateral sclerosis consistently at the extreme end of long survival. However, such clinical findings cannot be reliably used prognostically in isolation. A quantifiable prognostic measure might also be used to stratify patients within clinical trials to detect meaningful benefit in the shortest time. Knowledge of an individual patient's course at the time of diagnosis would facilitate effective care planning and resource targeting.

Finally, biomarkers sensitive to disease activity are the most needed. At present, clinical trials in ALS generally rely on tracheostomy-free survival, functional status such as the revised ALS Functional Rating Scale (ALSFRS-R), or both, as the primary outcome measures. Such trials are lengthy (12 – 18 months typically) and may be prohibitively

costly. A number of methods have been proposed to optimize and shorten such studies (6), but a robust, quantitative monitoring biomarker would have obvious value in this regard.

Biological themes

With the linking of familial ALS to several apparently disparate genes, it seems increasingly likely that ALS represents a common final endpoint. A deeper understanding of the common molecular pathways involved in ALS pathogenesis would facilitate the identification of biomarkers. Three genes linked to familial ALS in recent years, namely *TARDBP, FUS* and *C9orf72,* indicate a potentially pivotal role for RNA mis-processing in the pathogenesis of ALS (see Al-Chalabi et al., companion paper). Downstream of these events, cellular biological studies over the last two decades have led to the development of multiple interconnected pathogenic themes in ALS (7).

Excitotoxicity

Excitotoxicity encapsulates a process of neuronal death ultimately mediated by cellular calcium influx, triggered via excessive stimulation of receptors for glutamate. The finding of raised levels of glutamate in the CSF in ALS (8), with a sensitivity of only 40% in a later study and correlation with disease severity (9), provides support for this.

The astrocytic glutamate transporter EAAT2 is responsible for the clearance of synaptic glutamate, and its knockout in transgenic mice results in neuronal death (10). Reduced levels of spinal cord EAAT2 protein have been noted in end-stage rodent transgenic models of ALS (11,12), with abnormalities of EAAT2 protein expression demonstrable in up to 80% of human post mortem brain and spinal cord tissue (13). EAAT2 receptor dysfunction has been directly linked to mutant (but not wild-type) SOD1 in the presence of hydrogen peroxide (14). Overexpression of EAAT2 in mutant *SOD1* transgenic mice then delayed the onset of motor deficits (15, 16). This role for astrocytes supports the view that motor neuron degeneration in ALS is not cell-autonomous. Although the exact mechanism of action is uncertain, the only disease-modifying drug in ALS to date, namely riluzole, appears to have broadly anti-glutamatergic activity (17).

Glutamate acts through inotropic (NMDA, AMPA and kainate) and metabotropic receptors (MGluRs), and the relative contribution of these to. ALS pathogenesis remains uncertain. The development of novel selective positron emission tomography (PET) ligands may provide clarification. It is possible that excitotoxicity arises in ALS as a result of wider ‘upstream’ impairments of energy metabolism in ALS so that ‘normal’ levels of glutamate become toxic. There is also a body of evidence, including from in vivo neuroimaging, indicating an overall loss of neuronal inhibitory influence in ALS, perhaps through a primary interneuronal dysfunction, resulting in excitotoxicity through less balanced glutamatergic activity (18).

Oxidative stress

Oxidative stress arises from an alteration in the balance between the generation of reactive oxygen species (ROS) and their removal, together with the ability of the biological system to remove or repair ROS induced damage. The cumulative effect of oxidative stress in aged

non-replicating neurons, may be one important factor that tips the balance of homeostatic control mechanisms from an ability to cope with a toxic insult such as the presence of a disease-causing mutation, into a vicious cycle of cellular injury culminating in neuronal death and the onset of neurodegeneration in middle or later life. Oxidative stress causes structural damage (including DNA (19)) and also changes in redox-sensitive signalling pathways. The initial interest in the role of oxidative stress in ALS (20) was given new impetus with the discovery that mutations in *SOD1*, which encodes a major anti-oxidant defence protein, accounted for approximately 20% of cases of familial ALS (21). It is clear that oxidative stress interacts with and potentially exacerbates other pathophysiological processes contributing to motor neuron injury, including excitotoxicity, mitochondrial impairment, protein aggregation, endoplasmic reticulum stress and alterations in signalling from astrocytes and microglia. Several potential markers of free radical damage have been found in the CSF and blood from ALS patients (reviewed in (22)), and also in urine (23).

Cellular ROS are generated as by-products of aerobic metabolism, predominantly due to leakage of electrons from the mitochondrial respiratory chain, but with contributions from other intracellular enzyme systems including xanthine oxidase and cytochrome P450. ROS that are initially formed such as superoxide and hydrogen peroxide may undergo further reaction to produce more potent oxidant species including peroxynitrite and hydroxyl radicals. Biochemical indices of oxidative damage to proteins, lipids and DNA in excessive quantities compared to controls, can be found in post mortem tissue from apparently sporadic and *SOD1* -related familial cases. Oxidative damage to RNA species has also been documented, adding to the evidence that alteration in mRNA processing is an important pathophysiological mechanism in ALS (24). Indices of oxidative damage are also present in cellular and murine models of *SOD1* -related ALS, and interestingly the SOD1 protein itself appears to be particularly susceptible to oxidative post-translational modification. The recent development of cellular models of mutant TDP-43-related ALS indicates that the presence of mutant TDP-43 also provokes oxidative stress within motor neuronal cell lines (25).

Sources of oxidative stress in ALS have been investigated most thoroughly in mutant SOD1 (mSOD1) models, where several aberrant oxidative reactions have been proposed. However, enzymatically inactive SOD1, depleted of copper loading, is still capable of causing motor neuron degeneration, and mSOD1 may cause oxidative stress by mechanisms beyond its own catalytic activity (26). mSOD1 within microglia increases superoxide production by NADPH oxidase (Nox) enzymes. SOD1 stabilizes Rac1-GTP in the activated Nox2 complex, and mutant SOD1 locks Rac1 into its active state, with resultant prolongation of ROS production (27). Nox2 expression is increased in mSOD1 mice and human ALS, and survival of SOD1-G93A mice is extended by knock-out of either Nox1 or Nox2. The transcription factor Nrf2 (nuclear erythroid-2-related factor2) is a master regulator of the anti-oxidant response and responds to oxidative stress by binding and up-regulating anti-oxidant response element genes. Recent evidence has emerged that Nrf2-ARE signalling may be dysregulated in models of SOD1-related ALS and in the CNS of ALS patients (28).

A meta-analysis of therapeutic interventions in the mSOD1 mice up to 2007 concluded that anti-oxidant therapies were the class of drug most effective at improving survival (29). In human ALS, anti-oxidants have not yet shown benefit, although the reported trials have

often been of suboptimal design, and new in vitro screening methods may be able to generate future candidates (30).

Mitochondrial dysfunction

Mitochondria are critical for cell survival, acting as an energy source of the cell, buffering intracellular calcium, and regulating apoptosis. Damage to mitochondria selectively within tissues affected in ALS has been widely observed, especially in inherited disease caused by mutations in SOD1. Mitochondrial abnormalities may be a trigger or a consequence of the neurodegenerative process and the precise mechanisms remain uncertain (31, 32). Mutant SOD1 is localized in mitochondria, and accumulates on the outer membrane and inside the intermembrane space (33). Mutant SOD1 and other mutant proteins, such as TDP-43, may cause mitochondrial dysfunction by affecting the expression of mitochondrial proteins involved in energy metabolism. Mitochondria can also be affected by external SOD1 interfering with signalling and transport of the organelles. Key surface components inhibited by misfolded mSOD1 include the following:

- the voltage-dependent anion channel (VDAC1), the conductance of which is inhibited (34);
- the translocase of the outer membrane (TOM) transport complex, which is responsible for the import into mitochondria of over 1000 proteins made in the cytoplasm (35);
- Bcl2, an anti-apoptotic protein normally associated with mitochondria (36).

As a consequence of mSOD1 damage, mitochondria are non-uniformly distributed along axons of motor neurons (37).

The selective association of mSOD1 with mitochondria in cells of affected tissues remains uncertain, as is whether differences in mitochondria or cytoplasmic components (e.g. chaperones) may be responsible for tissue-specific abnormalities of protein folding. Drugs targeting mitochondrial properties (e.g. calcium conductance, biogenesis, fission, fusion, or transport) might provide therapeutic benefit. Mitochondrial modulators are a new class of potential therapeutic. Olesoxime promotes survival of mutant motor neurons in vitro (38), possibly by binding to the mitochondria, targeting VDAC and the benzodiazepine receptor. However, a recent human phase III study of olesoxime in ALS proved negative. Dexpramipexole is a mitochondrial ‘membrane stabilizer’ (39, 40), but a human phase III study in ALS also proved negative.

Neuroinflammation

Inflammatory mechanisms and immune reactivity are hypothesized to play a role in the pathogenesis of ALS (41). In experimental models, the progression of ALS has been linked to microglial cell/ macrophage activation in the spinal cord. Studies in patients with ALS have found elevated markers of inflammation (CRP, interleukin-6 and 13, macrophage chemotactic protein-1 (MCP-1)) (42). Levels of MCP-1 and other chemokines have also been detected in the CSF of ALS patients (43, 44). Such proteins may contribute to the amplification or possibly initiation of inflammation during ALS. Additionally, systemic

macrophage activation and alteration of macrophage surface markers have been linked to disease progression (45). Donor macrophages were present at sites of neuron loss after bone marrow transplantation, suggesting an ongoing migration of blood monocytes in patients with ALS (46). Macrophage activation markers in ALS blood are similar to those identified in the blood of patients with AIDS dementia where macrophages invading the CNS have been proven to induce neurodegeneration. Studies with Neuraltus Pharmaceuticals NP001, a small molecule regulator of macrophage activation in the *SOD1* mouse model, demonstrated longer survival compared with controls (19 days, p= 0.01) and slowing of the decline in neurological score. Phase II studies in humans are currently underway.

Altered energy metabolism

Studies in animal models have convincingly demonstrated that whole body energy physiology is impaired in ALS, and that this contributes to motor neuron degeneration (47, 48). In patients, much of the evidence that similar events occur is correlative. Most importantly, body mass index and overall nutritional status at disease onset appear to be strong predictors of survival of patients (49, 50). Circulating blood lipids are positively correlated with survival (51, 52) or functional status (53). Whether these statistical associations might translate into a sensitive and specific biomarker awaits further investigation. Multiple confounders might blur the sensitivity and specificity of such markers. first, dysphagia as a consequence of bulbar involvement, has a strong impact on nutritional status, and is on its own a sign of poorer prognosis. Secondly, impaired glucose tolerance has been observed in ALS patients, and it is unknown whether abnormalities in glucose metabolism influence survival in ALS. finally, regional and national dietary specificities are very likely to have strong influence on blood lipids and nutrition, and may confound the observed effects. Metabolomic studies in the blood of ALS patients and animal models might delineate a core set of metabolites that could be useful as biomarkers. A recent metabolomics study identified altered metabolites indicative of disrupted mitochondrial function and increased carbohydrate and lipid metabolism in ALS patients (54). Alternatively, imaging methods, in particular MRI of adipose tissues (55), DEXA-scan or CT may help to determine whether energy stores provide a biomarker related to energy metabolism.

Disease models

For human neurodegenerative diseases it is not currently possible to study cellular pathological processes in real time, or safely and repeatedly remove tissue for analysis. The ability to study cellular molecular processes, identify key pathways for intervention, and assess multiple candidate therapies over short periods of time, depends on the development of disease models. The ability to express both wild-type and mutant human genes in non-human cells and transgenic animals has provided a variety of possibilities for modelling many neurodegenerative disorders, including ALS (56, 57). The discovery of linkage to the *SOD1* gene of some cases of familial ALS led rapidly to the creation of an overexpressing mutant *SOD1* transgenic mouse (58). This and other models are summarized in Table II. Such models do not, as yet, capture pathogenesis at a systems level, and no one model has yet been able to reproduce all of the pathological and behavioural features of ALS.

Nonetheless, they have provided a valuable platform for testing many of the pathogenic hypotheses outlined earlier in this article, with hope for the future development of assays for high-throughput screening of therapeutic candidates.

Induced pluripotent stem cells

The generation of motor neurons from induced pluripotent stem cells (iPSCs), in turn derived from the skin fibroblasts of ALS patients (59), has marked a major advance in modelling pathogenesis in ALS, with potential for high-throughput therapeutic assessments. Encapsulated by the phrase ‘disease in a dish’, iPSCs carrying the *TDP-43* ‘M337V’ mutation have already been shown to reproduce several key aspects of TDP-43-related proteinopathies, including aggregate formation and reduced cell survival (60). The ability to generate not only motor neurons but also other non-neuronal cell types (including astrocytes, oligodendrocytes, microglia), will allow for versatility in teasing out cell-specific contributions to disease development. iPSCs from patients with familial ALS have already been made available to researchers at the NINDS Repository, thus allowing for the study of ALS arising from numerous *SOD1, FUS,* and *FIG4* mutations.

However, a number of challenges remain with regard to human iPSC uses for investigation into ALS biology. Currently, the long periods of time required to generate human neural cells (for example, astrocytes) results in significant expense and often complex experimental paradigms. Because iPSCs are derived from individual patients, careful assessment of the sample sizes studied should be considered before broad conclusions can be made about disease from a single iPSC source. The impact of the patients' age on the resulting iPSC remains unclear. If used to screen for potentially relevant ALS drug targets, standardization of cell lines across laboratories will be important for validation of drug effects. Future aims include the development of more complex integrated structures, for example the neuromuscular junction using muscle and neuronal cell cocultures (61).

Despite these challenges, it is likely that more efficient derivation of iPSC will be developed, thus shortening the time and expense currently associated with creating iPSC lines. As sources of iPSC become more readily available to the research community, investigators with a wide range of research interests will be able to draw on a range of ALS genotypes and phenotypes thus allowing for the identification of new ALS relevant disease targets for therapeutics.

Tissue and fluid biomarker sources

Biofluids

The range of human biofluids useful in proteomic studies to identify an ALS biomarker ‘signature’, includes CSF, blood, urine, and saliva (62). CSF is an excellent biofluid for biomarker discovery due to its approximation to the cells and brain and spinal cord regions exhibiting cell death during ALS. Blood, while more accessible, has greater protein complexity with greatly reduced concentrations, compared to CSF, of those proteins fundamentally involved in neuronal function.

During the past decade there has been a large increase in the number of ALS biomarker studies using CSF as well as blood (22). Most of these studies have examined changes of individual proteins in the CSF of ALS versus healthy control or other neurologic disease subjects, typically using a gel-based system or ELISA (63). However, most are limited by the number of samples used in the analysis, choice of control subjects, and typically the lack of verification in a separate cohort of patients. A number of more recent studies have used mass spectrometry or cytokine profiling to identify panels of candidate protein biomarkers in the CSF (43,64). Metabolomic approaches have been used to explore metabolic differences between ALS and control subjects in CSF and serum (54, 65 – 67). Early changes in patient metabolism and protein levels or post-translational modifications offer a means to both identify ALS at an early stage and to develop biomarkers to follow during disease progression.

At present, CSF candidate biomarkers in ALS can be grouped into those that reflect neuronal loss and those indicative of neuroinflammatory (glial) processes. For the former, neurofilaments have been the most reproducible (68 – 70). CSF TDP-43 appears to fall with disease progression in typical ALS (71), with lower values found in cases of ALS-FTD (72). The significance of finding reduced CSF transthyretin and cystatin C in ALS is uncertain (64). The latter is the essential constituent of the ‘pathognomonic’ Bunina body inclusion seen histopathologically in ALS and has been detected at increased levels in ALS plasma (73).

While several molecules linked to neuroinflammatory pathways have been reported as increased in ALS patient CSF, each has limited sensitivity in isolation and many studies have not been reproduced. A panel of cytokines, specifically interleukins 2, 6, 10, 15 and granulocyte-monocyte colony stimulating factor (GM-CSF) was nearly 90% accurate in distinguishing ALS patients from healthy controls (43). Other studies have also supported the concept of combining markers to improve accuracy, most recently the ratio of CSF phosphorylated neurofilament-heavy chain and complement C3, achieving even higher accuracy (74). CSF based biomarkers may be useful to aid in the diagnosis of ALS, but may have better applications in monitoring drug effects in clinical trials and as prognostic indicators of disease (73,75). Further studies, involving samples from mimic disorders rather than healthy controls, and serial samples from ALS patients, are required to validate the candidate diagnostic biomarkers and fully explore their utility in therapeutic trials. An important step has been to both recognize the potential variability in sample quality due to differences in acquisition and storage (76), and to establish international consensus on standard operating procedures (77).

Muscle

Skeletal muscle may represent a valuable source of biomarkers in ALS. This tissue is one of the most severely affected by the disease, with progressive denervation and atrophy, and it is easily accessible to biopsy. The only muscle biomarker that has been tested prospectively is the axon repellant Nogo-A. Nogo-A is strongly expressed in ALS muscles, is correlated with ALSFRS and prospectively identifies patients affected with lower motor neuron disease that will progress to ALS (78–80). However, the specificity of this increased expression has been

questioned. A combination of biomarkers might solve the problem of specificity. Muscle transcriptome analyses have identified a number of potential candidates correlated with disease severity (81). There are two potential limitations of muscle biomarkers. first, muscle biopsy is invasive and as a consequence longitudinal studies are very difficult. Secondly, muscle beds are very differentially involved among patients. The different sites of onset, as well as the heterogenous spreading, make it difficult to standardize the choice of the site of muscle biopsy. In this respect, the future of muscle biomarkers might be to identify a set of muscle derived proteins or peptides that enter the circulation. Such muscle-derived blood biomarkers could be useful tools to evaluate disease progression and severity in ALS patients in a manner similar to creatine kinase for myopathies.

Skin

The skin is an acknowledged part of the disease process in ALS, both in experimental models (82) and humans (83 – 85). Because this organ is easily accessible and at least some of the biochemical alterations are related to findings in the CNS (82, 83), the skin is a principal resource of biomarkers for diagnosis, staging, and evaluation of therapy. The most striking finding is an apparently selective elevation of the MMP-9 in the skin and spinal cord of experimental animals and in human skin and the CSF (82, 83). These findings are consistent with previous observations of increased collagen degradation, in particular collagen types I and IV, in the skin of ALS patients (84). The pathogenic steps responsible for these observations remain to be elucidated; however, free radical damage and inflammation are possible mechanisms. A further discovery was that small distal epidermal nerve fibers are affected in ALS (85), indicating the presence of a small fiber neuropathy. The pattern of changes reflects the concept of a distal axonopathy and paves the way for mechanistic studies of cytoskeletal alterations and axonal transport.

Post mortem tissues

While post mortem spinal cord, brain or muscle tissue may not provide the optimal resource for biomarker discovery efforts, they are especially important to help identify the cell types that express each candidate biomarker, as well as their relationship to the pathophysiology of the human disease. Increased efforts to collect ALS and appropriate control post mortem tissues become more crucial as the multitude of candidate biomarkers identified in the above mentioned biofluids and tissue biopsies will require proper characterization and correlation to ALS pathology. In addition, recent studies have shown that neural progenitor cells can be cultured from ALS post mortem tissues and used to generate astrocytes to investigate astrocyte derived cell signalling that influences motor neuron survival (86). Given the low numbers of banked ALS tissues at any one site, standardized collection and storage procedures for ALS post mortem tissues must be established to permit use of tissues obtained across multiple sites.

Neurophysiology biomarker sources

EMG and MUNE

Neurophysiological testing has been an important component of the diagnostic evaluation of patients with motor neuron disease since the demonstration that axon loss and consequent

reinnervation could be measured using electromyography (EMG) (87). While classical EMG and nerve conduction studies are still incorporated into diagnostic criteria for ALS (El Escorial), these techniques have not proved effective in monitoring disease progression or assessing effects of treatment. Motor unit number estimation (MUNE) is a tool that was developed for this purpose. The technique and theoretical basis for MUNE is quite simple; a maximum response amplitude, which is generated by activation of all motor units in the muscle, is recorded, from which an estimate of individual motor unit number is generated by dividing the maximum response amplitude by an estimate of single motor unit amplitude. Many techniques for estimating the average amplitude of single motor units have been suggested; most have been limited by sampling bias, and lack of reproducibility (88).

Recently, a modification of earlier described techniques was introduced and studied in a natural history study of patients with ALS (89). Multipoint incremental MUNE was found to have excellent test-retest reliability, and to decline monotonically in ALS faster than other measures traditionally used in ALS clinical trials. The technique can be well standardized, is performed briefly with good patient tolerance, and has a low computational burden. Using rate of decline as well as variability, hypothetical power analyses suggested that this measure might reduce both sample size and study duration in phase II trials. Other new MUNE methods, including MUNIX (90) and Bayseian MUNE (91,92) are also being developed. While no physiological method of motor unit number estimation has been validated against anatomical motor unit counts, their use as potential endpoints in clinical trials shows great promise.

Transcranial magnetic stimulation

ALS is diagnosed clinically by the presence of both UMN and LMN damage. While LMN abnormalities can be confirmed objectively using peripheral electrodiagnostic methods, UMN findings lack comparable established objective markers. Transcranial magnetic stimulation (TMS) is a neurophysiological test that measures UMN functional integrity, and can detect abnormalities when there are no clinical UMN signs. TMS works by evoking compound motor potentials (MEP) using non-invasive magnetic stimulation of the motor cortex through activation of both UMN and LMN pathways. It is used to study the conductivity and excitability of the corticospinal system. As potential physiological biomarkers, TMS measurements reflect the functional integrity of the UMN (93).

Single pulse TMS is a well established diagnostic and clinical research tool, although results vary widely between medical centers. However, if individual laboratories establish their own normative data, subjects can be tested in a consistent, reliable fashion. TMS recordings include measurements of motor threshold, central motor conduction time, and MEP amplitudes, all of which may be abnormal in ALS (94). TMS also can be delivered in paired pulses, or as repetitive trains of stimulation for investigating human brain function, transiently stimulating or inhibiting different cortical areas (95, 96).

TMS biomarkers, particularly single pulse evoked TMS amplitude, have been used to objectively discriminate ALS from controls and assess the progression of ALS (97 – 99). TMS can reveal subtle subclinical UMN dysfunction to help make the diagnosis of ALS as

well as clarify the relationship between ALS and its variants (100), including progressive muscular atrophy where there may be subclinical UMN changes.

Electrical impedance myography

Electrical impedance myography (EIM) is a technique in which a high-frequency, low-intensity electrical current is applied to a localized area of muscle and the consequent surface voltages measured (101). Unlike standard electromyography, in which the intrinsic electrical activity of muscle is measured, EIM assesses the integrity and structure of the muscle. An initial, single-center study demonstrated EIM's potential power at measuring disease progression in this disease (102). Recently, a second multicenter study compared EIM directly to the ALSFRS-R, MUNE, and handheld dynamometry (103). EIM outperformed the other measures in terms of its ability to detect deterioration. For example, based on these data, a study that would require 220 subjects using the ALSFRS-R would have the same power as one with only 95 using EIM. Studies in ALS rats have similarly showed a strong correlation to the rate of change in EIM and the animal's length of survival, as well as to MUNE (104). The methodology is also being applied in the first North American study of neural stem cells in ALS (105). One advantage of EIM over most other modalities is its ability to assess a variety of muscles and to measure specifically that area of the body where the disease is progressing most rapidly. Measurement of other muscles not routinely studied but which might provide valuable data, such as paraspinal muscles and the tongue, is also possible. Current efforts are geared toward further refining the technique for easy use and making it widely available (106).

Neuroimaging biomarker sources

MRI has a major role in the exclusion of cerebral, and particularly spinal mimics of ALS (107). However, neuroimaging is also at the forefront of advances in the understanding of in vivo cerebral disease mechanisms in ALS, including rodent models (108), with the identification of multiple candidate biomarkers as a result (109).

Radionuclide imaging

Single photon emission computed tomography (SPECT) is a practical and potentially widely applicable form of radionuclide imaging. It was at the forefront of the recognition of a clinical, pathological and, most recently, genetic continuum between ALS and frontotemporal dementia (FTD) (110). Positron emission tomography (PET) has greater resolution than SPECT but is limited by the availability of experienced facilities. Pivotal ‘activation’ PET studies, using tracers sensitive to blood flow and metabolism (e.g. radiolabelled water and flurodeoxyglucose, FDG), provided in vivo evidence for a consistent extramotor cerebral pathology in ALS (111). Subsequently, ‘ligand’ PET has been used to identify specific cerebral neuronal receptor changes in ALS. Such studies have provided evidence for a loss of cortical inhibition (112) that might influence progression rate (113), widespread microglial activation (114), and a striking reduction in serotonin-1A receptor binding (115), similar to changes seen in FTD (116). The future value of PET in ALS will depend upon the development of ligands with relevance to pathogenic hypotheses, e.g.

glutamate receptors, and more specific neuroinflammatory or protein markers, e.g. TDP-43 (the challenge of intracellular penetration notwithstanding).

Magnetic resonance imaging (MRI)

The observation of corticospinal tract hyperintensity lacks sensitivity and specificity for the diagnosis of ALS. Routine clinical structural imaging of the brain has limited value as a source of biomarkers in ALS, and only through advanced analysis methods (117). The acquisition of high-resolution 3D T1-weighted images and the development of novel pulse sequences such as diffusion tensor imaging and functional MRI have greatest potential in this regard, and would ultimately be feasible to perform within the clinical environment (118).

Macroscopic atrophy of the motor cortex is not a consistent observation in ALS, although prominent in those with PLS. Sophisticated analysis of 3D T1-weighted structural images acquired in 3D, known as voxel-based morphometry (VBM), can reveal subtle changes in regional cerebral tissue. Several VBM studies have been performed in ALS, and meta-analysis showed the right precentral gyrus as consistently altered (119). Surface-based morphometry allows assessment of cortical thickness, and a study in ALS confirmed not only primary motor, but also extramotor, temporal cortical thinning in faster progressors (120).

Post mortem histopathological study first demonstrated widespread cerebral white matter tract damage in ALS (121) and this can now be detected non-invasively using diffusion tensor imaging (DTI). This advanced MRI technique is sensitive to the movement of water, normally directionally confined within neuronal tracts. The two main quantitative measures of loss of neuronal tract integrity are reduced fractional anisotropy (FA) and increased mean diffusivity. Related parameters such as increased radial diffusivity may specifically reflect secondary demyelination of tracts in ALS. DTI studies have shown consistently reduced FA in the corticospinal tract, particularly within the posterior limb of the internal capsule (PLIC) (122) and corpus callosum (123) of ALS patients. Targeted FA measurement at the PLIC may provide prognostic information (124). DTI measures may, however, be insufficiently sensitive to longitudinal change over less than six months (125), nor have sufficient discriminatory power as an isolated measurement (126).

MRI is unsurpassed in its spatial resolution of cerebral functional activity achieved non-invasively. Blood oxygenation level-dependent (BOLD) functional MRI (fMRI) studies of motor tasks in ALS patients confirmed the widened region of activation observed in PET studies. More recently, however, it is the study of the ‘resting state’ that provides novel insight into ALS as a ‘system failure’. Resting-state fMRI (R-fMRI) has demonstrated increased functional connectivity within the damaged ALS cortical network, with possible implications in relation to cortical inhibitory influences (127). The combination of structural and functional MRI measures in this study also provided much better separation of patients from healthy age-matched controls.

Magnetic resonance spectroscopy (MRS) is an application of MRI that permits quantification of cerebral tissue metabolites. It has consistently demonstrated reduced N-

acetylaspartate ratios (a non-specific marker of neuronal loss) in the motor cortex of ALS patients, and high-field studies also suggest a specific loss of GABA-ergic influence (128). Recent studies applied to the cervical spinal cord of pre-symptomatic carriers of pathological SOD1 gene mutations demonstrated metabolite changes more consistent with affected ALS patients rather than healthy non-gene carriers (129), suggesting that MRS may be particularly sensitive to pre-clinical changes. This offers the hope of capturing the very earliest events in individuals carrying genetic abnormalities associated with the development of ALS, with the possibility of wider translation to sporadic cases.

The challenge for neuroimaging biomarker candidates is to move beyond results based on group averages, to individual measurements. It seems likely that this will require multiple parameters. Further longitudinal studies are needed to assess the sensitivity of multimodal MRI to disease activity compared to clinical assessments such as ALSFRS.

Concluding remarks

Major advances in the understanding of the pathobiology of ALS have occurred over the last two decades with developments in molecular biology, immunocytochemistry, neurophysiology and neuroimaging, and the recognition of overlap with some forms of FTD. It seems increasingly likely that there are multiple, possibly more discrete, pathways converging on motor neuron death. While recent discoveries in relation to RNA biology hint at a massively under-estimated level of pathological complexity in ALS, the common theme of misfolded protein inclusions across the range of neurodegenerative disorders brings the hope of a common strategy for the treatment of pre-aggregation events (130) and for common biomarker development. Biomarker candidates are emerging with the potential to refine the diagnosis, stratify patients prognostically, and facilitate therapeutic development. A key aim for further biomarker development, beyond validation across multiple centers, is the routine incorporation of biomarker measurement into future clinical trials.

Acknowledgments

MRT is supported by the Medical Research Council and Motor Neurone Disease Association UK Lady Edith Wolfson Fellowship. PJS is supported by the MND Association, the Medical Research Council and the European Community 7th Framework Programme.

References

1. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, et al. Amyotroph Lateral Scler. Lancet. 2011; 377:942–55. [PubMed: 21296405]
2. Mackenzie IR, Bigio EH, Ince PG, Geser F, Neumann M, Cairns NJ, et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. Ann Neurol. 2007; 61:427–34. [PubMed: 17469116]
3. Mitchell JD, Callagher P, Gardham J, Mitchell C, Dixon M, Addison-Jones R, et al. Timelines in the diagnostic evaluation of people with suspected amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND): a 20-year review. Can we do better? Amyotroph Lateral Scler. 2010; 11:537–41. [PubMed: 20565332]
4. Turner MR, Parton MJ, Shaw CE, Leigh PN, Al-Chalabi A. Prolonged survival in motor neuron disease: a descriptive study of the King's database 1990 – 2002. J Neurol Neurosurg Psychiatry. 2003; 74:995–7. [PubMed: 12810805]

5. Chio A, Calvo A, Moglia C, Mazzini L, Mora G. Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. J Neurol Neurosurg Psychiatry. 2011; 82:740–6. [PubMed: 21402743]
6. Cudkowicz ME, Katz J, Moore DH, O'Neill G, Glass JD, Mitsumoto H, et al. Toward more efficient clinical trials for amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2010; 11:259–65. [PubMed: 19961263]
7. Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Ann Neurol. 2009; 65(Suppl 1):S3–9. [PubMed: 19191304]
8. Rothstein JD, Tsai G, Kuncl RW, Clawson L, Cornblath DR, Drachman DB, et al. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. Ann Neurol. 1990; 28:18–25. [PubMed: 2375630]
9. Spreux-Varoquaux O, Bensimon G, Lacomblez L, Salachas F, Pradat PF, Le Forestier N, et al. Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: a reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. J NeurolvSci. 2002; 193:73–8.
10. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, et al. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron. 1996; 16:675–86. [PubMed: 8785064]
11. Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, et al. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron. 1997; 18:327–38. [PubMed: 9052802]
12. Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, et al. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc Natl Acad Sci U S A. 2002; 99:1604–9. [PubMed: 11818550]
13. Rothstein JD, van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol. 1995; 38:73–84. [PubMed: 7611729]
14. Trotti D, Rolfs A, Danbolt NC, Brown RH Jr, Hediger MA. SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. Nat Neurosci. 1999; 2:848. [PubMed: 10461226]
15. Guo H, Lai L, Butchbach ME, Stockinger MP, Shan X, Bishop GA, et al. Increased expression of the glial gluta-mate transporter EAAT2 modulates excitotoxicity and delays the onset but not the outcome of ALS in mice. Hum Mol Genet. 2003; 12:2519–32. [PubMed: 12915461]
16. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature. 2005; 433:73–7. [PubMed: 15635412]
17. Doble A. The pharmacology and mechanism of action of riluzole. Neurology. 1996; 47(Suppl 4):S233–41. [PubMed: 8959995]
18. Turner MR, Kiernan MC. Does interneuronal dysfunction contribute to neurodegeneration in amytrophic lateral sclerosis? Amyotroph Lateral Scler. 2012; 13:245–50. [PubMed: 22424125]
19. Bogdanov M, Brown RH, Matson W, Smart R, Hayden D, O'Donnell H, et al. Increased oxidative damage to DNA in ALS patients. Free Radic Biol Med. 2000; 29:652–8. [PubMed: 11033417]
20. Simpson EP, Yen AA, Appel SH. Oxidative stress: a common denominator in the pathogenesis of amyotrophic lateral sclerosis. Current Opinion in Rheumatology. 2003; 15:730–6. Epub 2003/10/22. [PubMed: 14569202]
21. Barber SC, Shaw PJ. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. Free Radic Biol Med. 2010; 48:629–41. [PubMed: 19969067]
22. Turner MR, Kiernan MC, Leigh PN, Talbot K. Biomarkers in amyotrophic lateral sclerosis. Lancet Neurol. 2009; 8:94–109. [PubMed: 19081518]
23. Mitsumoto H, Santella RM, Liu X, Bogdanov M, Zipprich J, Wu HC, et al. Oxidative stress biomarkers in sporadic ALS. Amyotroph Lateral Scler. 2008; 9:177–83. [PubMed: 18574762]
24. Chang Y, Kong Q, Shan X, Tian G, Ilieva H, Cleveland DW, et al. Messenger RNA oxidation occurs early in disease pathogenesis and promotes motor neuron degeneration in ALS. PLoS One. 2008; 3:2849.

25. Duan W, Li X, Shi J, Guo Y, Li Z, Li C. Mutant TAR DNA-binding protein-43 induces oxidative injury in motor neuron-like cell. Neuroscience. 2010; 169:1621–9. [PubMed: 20600671]
26. Kirby J, Halligan E, Baptista MJ, Allen S, Heath PR, Holden H, et al. Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS. Brain. 2005; 128:1686–706. [PubMed: 15872021]
27. Harraz MM, Marden JJ, Zhou W, Zhang Y, Williams A, Sharov VS, et al. SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model. J Clin Invest. 2008; 118:659–70. [PubMed: 18219391]
28. Sarlette A, Krampfl K, Grothe C, Neuhoff N, Dengler R, Petri S. Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 2008; 67:1055–62. [PubMed: 18957896]
29. Orrell RW, Lane RJ, Ross M. A systematic review of anti-oxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. Amyotroph Lateral Scler. 2008; 9:195–211. [PubMed: 18608090]
30. Barber SC, Higginbottom A, Mead RJ, Barber S, Shaw PJ. An in vitro screening cascade to identify neuroprotective anti-oxidants in ALS. Free Radic Biol Med. 2009; 46:1127–38. [PubMed: 19439221]
31. Magrane J, Manfredi G. Mitochondrial function, morphology, and axonal transport in amyotrophic lateral sclerosis. Antioxid Redox Signal. 2009; 11:1615–26. [PubMed: 19344253]
32. Kawamata H, Manfredi G. Mitochondrial dysfunction and intracellular calcium dysregulation in ALS. Mech Ageing Dev. 2010; 131:517–26. [PubMed: 20493207]
33. Kawamata H, Manfredi G. Different regulation of wild-type and mutant Cu/Zn superoxide dismutase localization in mammalian mitochondria. Hum Mol Genet. 2008; 17:3303–17. [PubMed: 18703498]
34. Israelson A, Arbel N, Da Cruz S, Ilieva H, Yamanaka K, Shoshan-Barmatz V, et al. Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS. Neuron. 2010; 67:575–87. [PubMed: 20797535]
35. Li Q, van de Velde C, Israelson A, Xie J, Bailey AO, Dong MQ, et al. ALS-linked mutant superoxide dismutase-1 (SOD1) alters mitochondrial protein composition and decreases protein import. Proc Natl Acad Sci U S A. 2010; 107:21146–51. [PubMed: 21078990]
36. Pedrini S, Sau D, Guareschi S, Bogush M, Brown RH Jr, Naniche N, et al. ALS-linked mutant SOD1 damages mitochondria by promoting conformational changes in Bcl-2. Hum Mol Genet. 2010; 19:2974–86. [PubMed: 20460269]
37. van de Velde C, McDonald KK, Boukhedimi Y, McAlonis-Downes M, Lobsiger CS, Bel Hadj S, et al. Misfolded SOD1 associated with motor neuron mitochondria alters mitochondrial shape and distribution prior to clinical onset. PLoS One. 2011; 6:22031.
38. Bordet T, Buisson B, Michaud M, Drouot C, Galea P, Delaage P, et al. Identification and characterization of cholest-4-en-3-one, oxime (TRO19622), a novel drug candidate for amyotrophic lateral sclerosis. J Pharmacol Exp Ther. 2007; 322:709–20. [PubMed: 17496168]
39. Cudkowicz M, Bozik ME, Ingersoll EW, Mller R, Mitsumoto H, Shefner J, et al. The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. Nat Med. 2011; 17:1652–6. [PubMed: 22101764]
40. Alavian KN, Dworetzky SI, Bonanni L, Zhang P, Sacchetti S, Mariggio MA, et al. Effects of dexpramipexole on brain mitochondrial conductances and cellular bioenergetic efficiency. Brain Res. 2012; 1446:1–11. [PubMed: 22364637]
41. Philips T, Robberecht W. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. Lancet Neurol. 2011; 10:253–63. [PubMed: 21349440]
42. Zhang R, Gascon R, Miller RG, Gelinas DF, Mass J, Hadlock K, et al. Evidence for systemic immune system alterations in sporadic amyotrophic lateral sclerosis (SALS). J Neuroimmunol. 2005; 159:215–24. Epub 2005/01/18. [PubMed: 15652422]
43. Mitchell RM, Freeman WM, Randazzo WT, Stephens HE, Beard JL, Simmons Z, et al. A CSF biomarker panel for identification of patients with amyotrophic lateral sclerosis. Neurology. 2009; 72:14–9. [PubMed: 18987350]

44. Kuhle J, Lindberg RL, Regeniter A, Mehling M, Steck AJ, Kappos L, et al. Increased levels of inflammatory chemokines in amyotrophic lateral sclerosis. Eur J Neurol. 2009; 16:771–4. [PubMed: 19236470]
45. Zhang R, Miller RG, Gascon R, Champion S, Katz J, Lancero M, et al. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (SALS). J Neuroimmunol. 2009; 206:121–4. Epub 2008/11/18. [PubMed: 19013651]
46. Appel SH, Engelhardt JI, Henkel JS, Siklos L, Beers DR, Yen AA, et al. Hematopoietic stem cell transplantation in patients with sporadic amyotrophic lateral sclerosis. Neurology. 2008; 71:1326–34. Epub 2008/10/22. [PubMed: 18936424]
47. Dupuis L, Oudart H, Rene F, Gonzalez de Aguilar JL, Loeffler JP. Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. Proc Natl Acad Sci U S A. 2004; 101:11159–64. [PubMed: 15263088]
48. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol. 2011; 10:75–82. [PubMed: 21035400]
49. Paganoni S, Deng J, Jaffa M, Cudkowicz ME, Wills AM. Body mass index, not dyslipidemia, is an independent predictor of survival in amyotrophic lateral sclerosis. Muscle Nerve. 2011; 44:20–4. Epub 2011/05/25. [PubMed: 21607987]
50. Marin B, Desport JC, Kajeu P, Jesus P, Nicolaud B, Nicol M, et al. Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry. 2011; 82:628–34. Epub 2010/11/26. [PubMed: 21097551]
51. Dupuis L, Corcia P, Fergani A, Gonzalez de Aguilar JL, Bonnefont-Rousselot D, Bittar R, et al. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology. 2008; 70:1004–9. [PubMed: 18199832]
52. Dorst J, Kühnlein P, Hendrich C, Kassubek J, Sperfeld AD, Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. J Neurol. 2011; 258:613–7. [PubMed: 21128082]
53. Chio A, Calvo A, Ilardi A, Cavallo E, Moglia C, Mutani R, et al. Lower serum lipid levels are related to respiratory impairment in patients with ALS. Neurology. 2009; 73:1681–5. Epub 2009/11/18. [PubMed: 19917991]
54. Lawton KA, Cudkowicz ME, Brown MV, Alexander D, Caffrey R, Wulff JE, et al. Biochemical alterations associated with ALS. Amyotroph Lateral Scler. 2012; 13:110–8. [PubMed: 22117131]
55. Müller HP, Raudies F, Unrath A, Neumann H, Ludolph AC, Kassubek J. Quantification of human body fat tissue percentage by MRI. NMR Biomed. 2011; 24:17–24. Epub 2010/07/31. [PubMed: 20672389]
56. Joyce PI, Fratta P, Fisher EM, Acevedo-Arozena A. SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mamm Genome. 2011; 22:420–48. [PubMed: 21706386]
57. Gama Sosa MA, de Gasperi R, Elder GA. Modeling human neurodegenerative diseases in transgenic systems. Hum Genet. 2012; 131:535–63. [PubMed: 22167414]
58. Gurney ME, Pu H, Chiu AY, dal Canto MC, Polchow CY, Alexander DD, et al. Motor neuron degeneration in mice that express a human Cu/Zn superoxide dismutase mutation. Science. 1994; 264:1772–5. [PubMed: 8209258]
59. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science. 2008; 321:1218–21. [PubMed: 18669821]
60. Bilican B, Serio A, Barmada SJ, Nishimura AL, Sullivan GJ, Carrasco M, et al. Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability. Proc Natl Acad Sci U S A. 2012; 109:5803–8. [PubMed: 22451909]
61. Thomson SR, Wishart TM, Patani R, Chandran S, Gillingwater TH. Using induced pluripotent stem cells (iPSC) to model human neuromuscular connectivity: promise or reality? J Anat. 2012; 220:122–30. [PubMed: 22133357]
62. Hu S, Loo JA, Wong DT. Human body fluid proteome analysis. Proteomics. 2006; 6:6326–53. [PubMed: 17083142]

63. Ryberg H, Bowser R. Protein biomarkers for amyotrophic lateral sclerosis. Expert Rev Proteomics. 2008; 5:249–62. [PubMed: 18466055]
64. Ranganathan S, Williams E, Ganchev P, Gopalakrishnan V, Lacomis D, Urbinelli L, et al. Proteomic profiling of cerebrospinal fluid identifies biomarkers for amyotrophic lateral sclerosis. J Neurochem. 2005; 95:1461–71. [PubMed: 16313519]
65. Blasco H, Corcia P, Moreau C, Veau S, Fournier C, Vourc'h P, et al. 1H-NMR-based metabolomic profiling of CSF in early amyotrophic lateral sclerosis. PLoS One. 2010; 5:13223.
66. Kumar A, Bala L, Kalita J, Misra UK, Singh RL, Khetrapal CL, et al. Metabolomic analysis of serum by (1)H NMR spectroscopy in amyotrophic lateral sclerosis. Clin Chim Acta. 2010; 411:563–7. [PubMed: 20096678]
67. Wuolikainen A, Moritz T, Marklund SL, Antti H, Andersen PM. Disease related changes in the cerebrospinal fluid metabolome in amyotrophic lateral sclerosis detected by GC/TOFMS. PLoS One. 2011; 6:17947.
68. Boylan K, Yang C, Crook J, Overstreet K, Heckman M, Wang Y, et al. Immunoreactivity of the phosphorylated axonal neurofilament H subunit (pNF-H) in blood of ALS model rodents and ALS patients: evaluation of blood pNF-H as a potential ALS biomarker. J Neurochem. 2009; 111:1182–91. [PubMed: 19765193]
69. Brettschneider J, Petzold A, Submuth SD, Ludolph AC, Tumani H. Axonal damage markers in cerebrospinal fluid are increased in ALS. Neurology. 2006; 66:852–6. [PubMed: 16567701]
70. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelso C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem. 1996; 67:2013–8. [PubMed: 8863508]
71. Kasai T, Tokuda T, Ishigami N, Sasayama H, Foulds P, Mitchell DJ, et al. Increased TDP-43 protein in cerebrospinal fluid of patients with amyotrophic lateral sclerosis. Acta Neuropathol. 2009; 117:55–62. [PubMed: 18989684]
72. Steinacker P, Hendrich C, Sperfeld AD, Jesse S, von Arnim CA, Lehnert S, et al. TDP-43 in cerebrospinal fluid of patients with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Arch Neurol. 2008; 65:1481–7. [PubMed: 19001167]
73. Wilson ME, Boumaza I, Lacomis D, Bowser R. Cystatin C: a candidate biomarker for amyotrophic lateral sclerosis. PLoS One. 2011; 5:15133.
74. Ganesalingam J, An J, Shaw CE, Shaw G, Lacomis D, Bowser R. Combination of neurofilament heavy chain and complement C3 as CSF biomarkers for ALS. J Neurochem. 2011; 117:528–37. Epub 2011/03/23. [PubMed: 21418221]
75. Sussmuth SD, Sperfeld AD, Hinz A, Brettschneider J, Endruhn S, Ludolph AC, et al. CSF glial markers correlate with survival in amyotrophic lateral sclerosis. Neurology. 2010; 74:982–7. [PubMed: 20308682]
76. Wuolikainen A, Hedenstrom M, Moritz T, Marklund SL, Antti H, Andersen PM. Optimization of procedures for collecting and storing of CSF for studying the metabolome in ALS. Amyotroph Lateral Scler. 2009; 10:229–36. [PubMed: 19412814]
77. Otto M, Bowser R, Turner M, Berry J, Brettschneider J, Connor J, et al. Roadmap and standard operating procedures for biobanking and discovery of neurochemical markers in ALS. Amyotroph Lateral Scler. 2012; 13:1–10. [PubMed: 22214350]
78. Dupuis L, Gonzalez de Aguilar JL, di Scala F, Rene F, de Tapia M, Pradat PF, et al. Nogo provides a molecular marker for diagnosis of amyotrophic lateral sclerosis. Neurobiol Dis. 2002; 10:358–65. [PubMed: 12270696]
79. Jokic N, Gonzalez de Aguilar JL, Pradat PF, Dupuis L, Echaniz-Laguna A, Muller A, et al. Nogo expression in muscle correlates with amyotrophic lateral sclerosis severity. Ann Neurol. 2005; 57:553–6. [PubMed: 15786457]
80. Pradat PF, Bruneteau G, Gonzalez de Aguilar JL, Dupuis L, Jokic N, Salachas F, et al. Muscle Nogo-A expression is a prognostic marker in lower motor neuron syndromes. Ann Neurol. 2007; 62:15–20. [PubMed: 17455292]
81. Pradat PF, Dubourg O, de Tapia M, di Scala F, Dupuis L, Lenglet T, et al. Muscle gene expression is a marker of amyotrophic lateral sclerosis severity. Neurodegener Dis. 2012; 9:38–52. Epub 2011/09/22. [PubMed: 21934272]

82. Fang L, Teuchert M, Huber-Abel F, Schattauer D, Hendrich C, Dorst J, et al. MMP-2 and MMP-9 are elevated in spinal cord and skin in a mouse model of ALS. J Neurol Sci. 2010; 294:51–6. [PubMed: 20441996]

83. Fang L, Huber-Abel F, Teuchert M, Hendrich C, Dorst J, Schattauer D, et al. Linking neuron and skin: matrix metal-loproteinases in amyotrophic lateral sclerosis (ALS). J Neurol Sci. 2009; 285:62–6. [PubMed: 19523650]

84. Ono S. The skin in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000; 1:191–9. [PubMed: 11464952]

85. Weis J, Katona I, Muller-Newen G, Sommer C, Necula G, Hendrich C, et al. Small-fiber neuropathy in patients with ALS. Neurology. 2011; 76:2024–9. [PubMed: 21646630]

86. Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, et al. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nat Biotechnol. 2011; 29:824–8. [PubMed: 21832997]

87. Erminio F, Buchthal F, Rosenfalck P. Motor unit territory and musclefiber concentration in paresis due to peripheral nerve injury and anterior horn cell involvement. Neurology. 1959; 9:657–71. [PubMed: 13820657]

88. Shefner JM, Gooch CL. Motor unit number estimation. Phys Med Rehabil Clin N Am. 2003; 14:243–60. [PubMed: 12795515]

89. Shefner JM, Watson ML, Simionescu L, Caress JB, Burns TM, Maragakis NJ, et al. Multipoint incremental motor unit number estimation as an outcome measure in ALS. Neurology. 2011; 77:235–41. [PubMed: 21676915]

90. Nandedkar SD, Barkhaus PE, Stalberg EV. Reproducibility of MUNIX in patients with amyotrophic lateral sclerosis. Muscle Nerve. 2011; 44:919–22. [PubMed: 21953206]

91. Henderson RD, Ridall PG, Pettitt AN, McCombe PA. Results of Bayesian statistical analysis in normal and ALS subjects. Suppl Clin Neurophysiol. 2009; 60:57–63. [PubMed: 20715367]

92. Henderson RD, Ridall PG, Hutchinson NM, Pettitt AN, McCombe PA. Bayesian statistical MUNE method. Muscle Nerve. 2007; 36:206–13. [PubMed: 17487869]

93. Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology. 2007; 68:484–8. [PubMed: 17296913]

94. Eisen A, Shytbel W, Murphy K, Hoirch M. Cortical magnetic stimulation in amyotrophic lateral sclerosis. Muscle Nerve. 1990; 13:146–51. [PubMed: 2314418]

95. Yokota T, Yoshino A, Inaba A, Saito Y. Double cortical stimulation in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 1996; 61:596–600. [PubMed: 8971106]

96. Weber M, Eisen A, Stewart HG, Andersen PM. Preserved slow conducting corticomotoneuronal projections in amyotrophic lateral sclerosis with autosomal recessive D90A Cu/Zn superoxide dismutase mutation. Brain. 2000; 123:1505–15. [PubMed: 10869061]

97. Mitsumoto H, Ulug AM, Pullman SL, Gooch CL, Chan S, Tang MX, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. Neurology. 2007; 68:1402–10. [PubMed: 17452585]

98. Attarian S, Vedel JP, Pouget J, Schmied A. Progression of cortical and spinal dysfunctions over time in amyotrophic lateral sclerosis. Muscle Nerve. 2008; 37:364–75. [PubMed: 18080998]

99. Floyd AG, Yu QP, Piboolnurak P, Tang MX, Fang Y, Smith WA, et al. Transcranial magnetic stimulation in ALS: utility of central motor conduction tests. Neurology. 2009; 72:498–504. [PubMed: 19204259]

100. Vucic S, Cheah BC, Yiannikas C, Kiernan MC. Cortical excitability distinguishes ALS from mimic disorders. Clin Neurophysiol. 2011; 122:1860–6. [PubMed: 21382747]

101. Rutkove SB. Electrical Impedance Myography: Background, Current State, and Future Directions. Muscle Nerve. 2009; 40:936–46. [PubMed: 19768754]

102. Rutkove SB, Zhang H, Schoenfeld DA, Raynor EM, Shefner JM, Cudkowicz ME, et al. Electrical impedance myography to assess outcome in amyotrophic lateral sclerosis clinical trials. Clin Neurophysiol. 2007; 118:2413–8. [PubMed: 17897874]

103. Rutkove SB, Caress JB, Cartwright MS, Burns TM, Warder J, David WS, et al. Electrical impedance myography as a biomarker to assess ALS progression. Amyotroph Lateral Scler. 2012; 13:439–45. [PubMed: 22670883]

104. Wang LL, Spieker AJ, Li J, Rutkove SB. Electrical impedance myography for monitoring motor neuron loss in the SOD1-G93A amyotrophic lateral sclerosis rat. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology. 2011; 122:2505–11. Epub 2011/05/27. [PubMed: 21612980]

105. Glass JD, Boulis NM, Johe K, Rutkove SB, Federici T, Polak M, et al. Lumbar intraspinal injection of neural stem cells in patients with amyotrophic lateral sclerosis: results of a phase I trial in 12 patients. Stem Cells. 2012; 30:1144–51. Epub 2012/03/15. [PubMed: 22415942]

106. Ogunnika OT, Rutkove SB, Ma H, Fogerson PM, Scharfstein M, Cooper RC, et al. A portable system for the assessment of neuromuscular diseases with electrical impedance myography. Journal of Medical Engineering & Technology. 2010; 35:377–85. Epub 2010/07/31. [PubMed: 20670104]

107. Filippi M, Agosta F, Abrahams S, Fazekas F, Grosskreutz J, Kalra S, et al. EFNS guidelines on the use of neuroimaging in the management of motor neuron diseases. Eur J Neurol. 2010; 17:520–6.

108. Evans MC, Modo M, Talbot K, Sibson N, Turner MR. Magnetic resonance imaging of pathological processes in rodent models of amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2012; 13:288–301. [PubMed: 22117132]

109. Turner MR, Agosta F, Bede P, Govind V, Lule D, Verstraete E. Neuroimaging in amyotrophic lateral sclerosis. Biomark Med. 2012; 6:319–37. [PubMed: 22731907]

110. Neary D, Snowden JS, Mann DM, Northen B, Goulding PJ, Macdermott N. Frontal lobe dementia and motor neuron disease. J Neurol Neurosurg Psychiatry. 1990; 53:23–32. [PubMed: 2303828]

111. Kew JJ, Leigh PN, Playford ED, Passingham RE, Goldstein LH, Frackowiak RS, et al. Cortical function in amyotrophic lateral sclerosis: a positron emission tomography study. Brain. 1993; 116:655–80. [PubMed: 8513396]

112. Lloyd CM, Richardson MP, Brooks DJ, Al Chalabi A, Leigh PN. Extramotor involvement in ALS: PET studies with the GABA(A) ligand [(11)C]flumazenil. Brain. 2000; 123:2289–96. [PubMed: 11050028]

113. Turner MR, Hammers A, Al-Chalabi A, Shaw CE, Andersen PM, Brooks DJ, et al. Distinct cerebral lesions in sporadic and D90A-SOD1 ALS: studies with [11C] flumazenil PET. Brain. 2005; 128:1323–9. [PubMed: 15843422]

114. Turner MR, Cagnin A, Turkheimer FE, Mller CC, Shaw CE, Brooks DJ, et al. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [(11)C](R)-PK11195 positron emission tomography study. Neurobiol Dis. 2004; 15:601–9. [PubMed: 15056468]

115. Turner MR, Rabiner EA, Hammers A, Al-Chalabi A, Grasby PM, Shaw CE, et al. [11C]-WAY100635 PET demonstrates marked 5-HT1A receptor changes in sporadic ALS. Brain. 2005; 128:896–905. [PubMed: 15689356]

116. Lanctot KL, Herrmann N, Ganjavi H, Black SE, Rusjan PM, Houle S, et al. Serotonin-1A receptors in frontotemporal dementia compared with controls. Psychiatry Res. 2007; 156:247–50. [PubMed: 17976961]

117. Ding XQ, Kollewe K, Blum K, Korner S, Kehbel S, Dengler R, et al. Value of quantitative analysis of routine clinical MRI sequences in ALS. Amyotroph Lateral Scler. 2011; 12:406–13. [PubMed: 21812629]

118. Turner MR, Grosskreutz J, Kassubek J, Abrahams S, Agosta F, Benatar M, et al. Towards a neuroimaging biomarker for amyotrophic lateral sclerosis. Lancet Neurol. 2011; 10:400–3. [PubMed: 21511189]

119. Chen Z, Ma L. Grey matter volume changes over the whole brain in amyotrophic lateral sclerosis: a voxel-wise meta-analysis of voxel based morphometry studies. Amyotroph Lateral Scler. 2010; 11:549–54. [PubMed: 20929296]

120. Verstraete E, Veldink JH, Hendrikse J, Schelhaas HJ, van den Heuvel MP, van den Berg LH. Structural MRI reveals cortical thinning in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2011; 83:383–8. [PubMed: 21965521]

121. Smith MC. Nervefibre degeneration in the brain in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 1960; 23:269–82. [PubMed: 21610893]

122. Li J, Pan P, Song W, Huang R, Chen K, Shang H. A meta-analysis of diffusion tensor imaging studies in amyotrophic lateral sclerosis. Neurobiol Aging. 2011; 33:1833–8. [PubMed: 21621298]

123. Filippini N, Douaud G, Mackay CE, Knight S, Talbot K, Turner MR. Corpus callosum involvement is a consistent feature of amyotrophic lateral sclerosis. Neurology. 2010; 75:1645–52. [PubMed: 21041787]

124. Menke RAL, Abraham I, Thiel CS, Filippini N, Knight S, Talbot K, et al. Fractional anisotropy in the posterior limb of the internal capsule and prognosis in amyotrophic lateral sclerosis. Arch Neurol. 2012; 69:1493–9. [PubMed: 22910997]

125. van der Graaff MM, Sage CA, Caan MW, Akkerman EM, Lavini C, Majoie CB, et al. Upper and extramotor neuron involvement in early motor neuron disease: a diffusion tensor imaging study. Brain. 2011; 134:1211–28. [PubMed: 21362631]

126. Foerster BR, Dwamena BA, Petrou M, Carlos RC, Callaghan BC, Pomper MG. Diagnostic Accuracy Using Diffusion Tensor Imaging in the Diagnosis of ALS: A Meta-analysis. Acad Radiol. 2012; 19:1075–86. [PubMed: 22749050]

127. Douaud G, Filippini N, Knight S, Talbot K, Turner MR. Integration of structural and functional magnetic resonance imaging in amyotrophic lateral sclerosis. Brain. 2011; 134:3470–9. [PubMed: 22075069]

128. Foerster BR, Callaghan BC, Petrou M, Edden RA, Chenevert TL, Feldman EL. Decreased motor cortex gamma-aminobutyric acid in amyotrophic lateral sclerosis. Neurology. 2012; 78:1596–600. [PubMed: 22517106]

129. Carew JD, Nair G, Andersen PM, Wuu J, Gronka S, Hu X, et al. Presymptomatic spinal cord neurometabolicfindings in SOD1-positive people at risk for familial ALS. Neurology. 2011; 77:1370–5. [PubMed: 21940617]

130. Polymenidou M, Cleveland DW. The seeds of neurodegeneration: prion-like spreading in ALS. Cell. 2011; 147:498–508. [PubMed: 22036560]

131. Douglass CP, Kandler RH, Shaw PJ, McDermott CJ. An evaluation of neurophysiological criteria used in the diagnosis of motor neuron disease. J Neurol Neurosurg Psychiatry. 2010; 81:646–9. [PubMed: 20522872]

132. Haverkamp LJ, Appel V, Appel SH. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. Brain. 1995; 118(Pt 3):707–19. [PubMed: 7600088]

133. Turner MR, Bakker M, Sham P, et al. Prognostic modelling of therapeutic interventions in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2002; 3:15–21. [PubMed: 12061944]

134. Cedarbaum JM, Stambler N, Malta E, et al. The ALS-FRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999; 169:13–21. [PubMed: 10540002]

135. Johnson BS, McCaffery JM, Lindquist S, Gitler AD. A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. Proc Natl Acad Sci U S A. 2008; 105:6439–44. [PubMed: 18434538]

136. Bastow EL, Gourlay CW, Tuite MF. Using yeast models to probe the molecular basis of amyotrophic lateral sclerosis. Biochem Soc Trans. 2011; 39:1482–87. [PubMed: 21936838]

137. Couthouis J, Hart MP, Shorter J, et al. A yeast functional screen predicts new candidate ALS disease genes. Proc Natl Acad Sci U S A. 2011; 108:20881–90. [PubMed: 22065782]

138. Oeda T, Shimohama S, Kitagawa N, et al. Oxidative stress causes abnormal accumulation of familial amyotrophic lateral sclerosis-related mutant SOD1 in transgenic Caenorhabditis elegans. Hum Mol Genet. 2001; 10:2013–23. [PubMed: 11590119]

139. Vaccaro A, Tauffenberger A, Aggad D, et al. Mutant TDP-43 and FUS cause age-dependent paralysis and neurodegeneration in C. elegans. PLoS One. 2012; 7:e31321. [PubMed: 22363618]

140. Lanson NA Jr, Maltare A, King H, et al. A Drosophila model of FUS-related neurodegeneration reveals genetic interaction between FUS and TDP-43. Hum Mol Genet. 2011; 20:2510–23. [PubMed: 21487023]

141. Wang JW, Brent JR, Tomlinson A, et al. The ALS-associated proteins FUS and TDP-43 function together to affect Drosophila locomotion and life span. J Clin Invest. 2011; 121:4118–26. [PubMed: 21881207]

142. Xia R, Liu Y, Yang L, et al. Motor neuron apoptosis and neuromuscular junction perturbation are prominent features in a Drosophila model of Fus-mediated ALS. Mol Neurodegener. 2012; 7:10. [PubMed: 22443542]

143. Miguel L, Avequin T, Delarue M, et al. Accumulation of insoluble forms of FUS protein correlates with toxicity in Drosophila. Neurobiol Aging. 2012; 33:1008 e1001–1015. [PubMed: 22118902]

144. Bosco DA, Lemay N, Ko HK, et al. Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. Hum Mol Genet. 2010; 19:4160–75. [PubMed: 20699327]

145. Kabashi E, Bercier V, Lissouba A, et al. FUS and TARDBP but not SOD1 interact in genetic models of amyotrophic lateral sclerosis. PLoS Genet. 2011; 7:e1002214. [PubMed: 21829392]

146. Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. Prog Neurobiol. 2008; 85:94–134. [PubMed: 18282652]

147. Awano T, Johnson GS, Wade CM, et al. Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2009; 106:2794–99. [PubMed: 19188595]

148. Uchida A, Sasaguri H, Kimura N, et al. Non-human primate model of amyotrophic lateral sclerosis with cytoplasmic mislocalization of TDP-43. Brain. 2012; 135:833–46. [PubMed: 22252998]

Table I

Types of biomarker in ALS, their value and the current gold standards.

Biomarker type	Value	Current benchmarks
Diagnostic	Initiate therapy earlier; Exclude ALS	Neurological history & examination Electromyography (Revised El Escorial/Awaji criteria) [131]
Prognostic	Identify patterns of progression: **1** Improved stratification in therapeutic trials **2** Timely intervention and optimal care e.g. gastrostomy, non-invasive ventilation, cognitive support	Diagnostic latency Neurological evaluation (e.g. clinical phenotypes) Cox modelling of clinical variables [132,133]
Monitoring	Identify ineffective drugs earlier	Revised ALS Functional Rating Score [134] (Electrical impedance myography emergent [102])

Table II

Models of ALS pathology, and their key features.

Model	Specifics	ALS-like pathology	Potentially relevant physical features	Advantages	Disadvantages	Key references
Yeast	*Saccharomyces cerevisiae SOD1, TARDBP,* or FUS-related transgenics; Also used as a screening model for novel RNA-binding-related genes	Mutant SOD1 disrupts mitochondrial homoeostasis; Recapitulation of TDP-43 & FUS aggregation with identification of modulators	NA	Readily available, low maintenance; homologous basic cellular structures to humans; non-animal; rapid turnover; high throughput	Cellular toxicity arising simply from over-expression of human proteins; facultative aerobe unlike human cells	135-137
Worm	*Caenorhabditis elegans SOD1, TARDBP,* or *FUS*-related transgenics	Increased sensitivity to oxidative stress in presence of mutant SOD1, with aggregate formation; Aggregates of mutant TDP-43 & FUS	Progressive 'incoordination' & paralysis over hours-days in overexpressing mutant (especially phosphorylated) > wild-type TDP-43 & FUS (and only mutant SOD1) worms	Readily available, low maintenance; homologous basic neuromuscular structures to humans	Variable effects and limited human clinical similarity	Reviewed in[56] See also[138,139]
Fruit fly	*Drosophlia melanogaster SOD1, TARDBP,* or *FUS*-related transgenics	Variable effects of both wild-type and mutant TDP-43 & FUS on aggregate formation, dendritic branching, and NMJ dysfunction depending on tissue expression	Variable effects of both wild-type and mutant SOD1, TDP-43 & FUS on larval locomotor function depending on tissue expression	Readily available, low maintenance; homologous basic neuromuscular structures to humans; short life cycle	Variable effects and limited human clinical similarity	Reviewed in[56] See also[140-143]
Zebrafish	*Danio rerio SOD1, TARDBP,* or *FUS*-related transgenics	Mutant SOD1 linked to MN loss and dysmorphic NMJs; Mutant TDP-43 linked to decreased motor axon length and branching; Mutant FUS linked to cytoplasmic inclusions	Mutant SOD1 associated with motor abnormalities and muscle atrophy	Readily available, low maintenance; homologous basic neuromuscular structures to humans; short life cycle	Variable effects and limited human clinical similarity	Reviewed in[56] See also[144,145]
Rodent	*Mus musculus* ≫ *Rattus norvegicus SOD1* or *TARDBP*-related transgenics	Mutant SOD1 linked to gliosis, ubiquitinated SOD1 inclusions, mitochondrial vacuolation, axonal and MN loss; WT and mutant TDP-43 more variably linked to cellular aggregates or MN loss	Progressive locomotor abnormalities with hind-limb weakness and muscle wasting from ~3 months WT and mutant TDP-43 less consistently linked to any motor abnormalities and muscle atrophy	Consistent motor phenotype; readily available, low maintenance; homologous basic neuromuscular structures to humans; short life cycle	Costly infrastructure; some ethical concerns Poor translation of therapeutic response in SOD1 mouse to human studies so far TDP-43 models show limited motor phenotype	Reviewed in[56,146]
Dog	'Canine degenerative myelopathy': Pembroke Welsh corgi, Boxer, Rhodesian ridgeback, German Shepherd, & Chesapeake Bay retriever all homozygous for *SOD1* 'E40K' missense mutation	Lateral cord white matter myelin and axonal loss; Neuronal cytoplasmic inclusions binding anti-SOD1 antibodies	Adult-onset progressive spastic myelopathy affecting pelvic girdle, leading to eventual flaccid quadriparesis	Similar to human SOD1-related ALS in being a delayed adult-onset disorder	Ethical concerns, availablity and infrastructure issues; long latency to symptoms; limited relevance to non-SOD1-related ALS	147
Monkey	*Macaca fascicularis* over-expressing human TDP-43	Cytoplasmic mislocalization of TDP-43; cystatin-C positive Aggregates	Progressive motor weakness of distal upper limbs with fasciculation and wasting	Closest species to humans physically and behaviourally	Major ethical concerns and infrastructure issues; limited relevance to	148

Model	Specifics	ALS-like pathology	Potentially relevant physical features	Advantages	Disadvantages	Key references
	via adenovirus to cervical cord					
	via adenovirus to cervical cord				‘slowly-developing’ human ALS	

Amyotroph Lateral Scler Frontotemporal Degener. Author manuscript; available in PMC 2015 January 05.