Quantifying Disease Progression in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) exhibits characteristic variability of onset and rate of disease progression, with inherent clinical heterogeneity making disease quantitation difficult. Recent advances in understanding pathogenic mechanisms linked to the development of ALS impose an increasing need to develop strategies to predict and more objectively measure disease progression. This review explores phenotypic and genetic determinants of disease progression in ALS, and examines established and evolving biomarkers that may contribute to robust measurement in longitudinal clinical studies. With targeted neuroprotective strategies on the horizon, developing efficiencies in clinical trial design may facilitate timely entry of novel treatments into the clinic.

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Amyotrophic lateral sclerosis (ALS) is characterized by heterogeneity in the region of onset, rate of progression, patterns of disease spread, and relative burden of upper motor neuron (UMN), lower motor neuron (LMN), and cognitive pathology. This phenotypic variability in ALS complicates measurement of disease progression. However, recent conceptual and technological advances have suggested novel approaches. With the dawning era of targeted therapeutics in ALS, accurate measurement of disease burden remains a critical priority to facilitate efficient clinical trial design and to enable further insights into disease pathogenesis. As such, the present review will discuss the current tools and future biomarker and clinical trial approaches that may be useful in measuring disease progression in ALS.

Clinical and Genetic Determinants of Progression

Recognized ALS Clinical Phenotypes

The clinical hallmark of ALS is the presence of concomitant UMN and LMN disease involving brainstem- and spinal-innervated regions. Disease onset in ALS is typically anatomically localized, with subsequent spread into other, usually contiguous body regions. Patterns of disease involvement and spread have been described,[1–4] which may facilitate anticipation of the sequence of regional involvement and prognosis. Predicting patterns of disease spread may be useful when measuring treatment response, and specific staging systems have been devised to account for regional spread in ALS.[5,6] In an individual with ALS, disease advances at a relatively constant rate,[7] although progression may be influenced by clinical, demographic, and genetic features (Table 1).

A number of distinct clinical phenotypes exist within the ALS disease spectrum, and may be associated with rates of disease progression that differ from those of more typical ALS (see Table 1). For example, flail-limb variant ALS presents with progressive LMN weakness of the upper limbs and may remain relatively confined for a prolonged period, resulting in a man-in-the-barrel appearance. Flail-limb variant, along with other LMN-predominant

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TABLE 1. Factors Influencing the Rate of Progression in ALS

Factor	Associated with Longer Survival	Associated with Shorter Survival
Phenotype	Flail limb variant,[9] LMN-predominant disease,[8] UMN-predominant disease,[82] prolonged interval to diagnosis[83]	Bulbar onset ALS,[2,84–86] respiratory onset ALS,[87] cognitive impairment,[88,89] impaired nutritional status,[90] neck flexor weakness[91]
Demographic features	Younger age at diagnosis[84,92]	Older age at diagnosis,[84,92] lower economic status,[93] smoking[92,94]
Genetic influences	E21G, G37R, D90A G93C, and I113T mutations in *SOD1*,[95] reduced *KIFAP3* gene expression,[96] reduced *EPHA4* gene expression[97]	A4V mutation in *SOD1,*[98] *FUS* mutations with basophilic inclusions[99]
Treatment	Riluzole,[85,93] noninvasive ventilation,[100] enteral feeding,[101] moderate exercise,[102] multidisciplinary clinic care[103]	Topiramate[104]

ALS = amyotrophic lateral sclerosis; EPHA4 = ephrin type-A receptor 4; FUS = fused in sarcoma; KIFAP3 = kinesin-associated protein 3; LMN = lower motor neuron; SOD1 = superoxide dismutase 1; UMN = upper motor neuron.

subtypes such as progressive muscular atrophy, may be characterized by slower disease progression.[8,9]

Patients may also present with UMN-predominant disease. On the extreme of this spectrum is primary lateral sclerosis (PLS), where UMN signs remain isolated, although eventually many patients evolve LMN features over time.[10] On average, UMN-predominant and LMN-predominant phenotypes have a better prognosis than classic ALS presentations, although within these groups there may still be marked variability in the rate of disease progression (see Table 1).

Some clinicians consider the different phenotypes as fitting within a clinical and pathological continuum (lumpers), whereas others suggest that variation in the clinical presentation may reflect heterogeneity of underlying pathophysiological mechanisms (splitters).[11] This issue of accurate disease categorization remains a subject of contention, in need of more complete exploration.

Influence of Genetic and Epidemiological Factors

The contribution of genetic factors to the clinical phenotype and rate of progression in ALS is incompletely understood. Except in a few cases, there is no obvious relationship between underlying genetic cause and phenotype (for a full record, see the ALS Online Genetic Database at http://alsod.iop.kcl.ac.uk; see Table 1).[12]

Several ALS genes exhibit a phenomenon called pleiotropy, where the same gene variation can result in different phenotypes.[13] For example, the *C9orf72* mutation is a pathological expansion of a repeated DNA sequence. In some individuals this results in ALS, but in others it causes frontotemporal dementia, ALS and frontotemporal dementia, or other less common phenotypes such as psychosis.[14] Penetrance has not been definitively established, and not everyone carrying the pathological expansion will develop disease during their lifetime. These observations suggest that environmental factors interact with the mutation to affect outcome. The resulting phenotype does show some correlation with survival, because individuals with cognitive impairment have a faster progression than those without.[15]

Historically, detection of genetic mutations contributing to ALS pathogenesis has been difficult, as ALS is relatively rare and cohorts of patients with a positive family history are small. Technological advances in genetic analysis, in particular next generation high-throughput sequencing (NGS), may further illuminate the role of genetic influences on ALS disease risk and progression.[16] In NGS, multiple sequences are produced in parallel, which improves the efficiency of the process and hence decreases time and cost. Presently, whole exome sequencing with NGS may be a cost-effective way of screening for genetic mutations in coding regions, both in patients with familial ALS without an identified mutation, and to identify clustering of genetic variations in sporadic ALS that may help clarify genetic contributions to disease progression. With increasing cost efficiencies, whole genome sequencing may provide a superior means of genetic screening.[17] Collaborative research efforts performing NGS of stored samples from existing cohorts of patients with clinical and progression data (for example via the PRO-ACT database[18]) may provide a mechanism to predict disease progression prospectively.

Reanalysis of completed treatment trials with more detailed genetic information may identify genetic influences on treatment efficacy.

The relationship between genotype, phenotype, environment, and prognosis has implications for clinical trials. Stratification into groups with homogeneous survival would improve statistical power, and might reveal treatments effective in one group that are not so in another. Until recently, stratification was only possible by phenotype, because no environmental factors were known and identified genetic causes were rare. The identification of pathological GGGGCC expansions in *C9orf72* in approximately 7% of individuals with sporadic ALS[17,19] may provide the opportunity to analyze this group separately, although variability in the phenotype and rate of progression remains an issue in patients with *C9orf72* mutation just as in sporadic ALS. As underlying genetic contributions to ALS are identified, such stratification will become easier.

Quantifying Clinical Progression

MEASURING SURVIVAL. Major treatment trials undertaken in ALS have focused on survival and clinical endpoints for efficacy analysis. As ALS remains a clinical diagnosis, clinical measurement strategies are intuitive as research endpoints. Regulatory approval of new therapies by the US Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products requires evidence of improvement of clinical endpoints such as survival, function, and strength measures. As such, reliable, sensitive, and broadly applicable clinical instruments for the monitoring of disease progression in ALS will remain important in clinical trial design (Table 2).

Improved survival, typically defined as survival without tracheostomy or permanent assisted ventilation, is clearly an important objective for a proposed treatment in ALS. However, obtaining meaningful change in these indices may prolong trial duration, increase sample size and cost, and be influenced by variation in respiratory intervention and end-of-life care at different institutions. Some ALS treatment trials have reported relatively few survival events, which may partly reflect patient selection bias. That is, end-stage patients, such as those with substantial respiratory involvement or those too unwell to attend to the requirements of trial follow-up, may not be referred to a trial center or may be ineligible for enrollment. Conversely, limiting trial entry to those patients with disease duration less than a specified cutoff, for example 24 months, may eliminate those patients with longer disease duration and slower progression. As such, patient selection factors may skew the phenotypes of included trial participants and thereby influence survival data.

FUNCTIONAL ASSESSMENTS. Survival measures may also be insensitive to potentially significant changes in functional status. All of the major trials in ALS have included a functional scale as a primary or secondary endpoint. The revised ALS Functional Rating Scale (ALSFRS-R) is most commonly used, and evaluates symptoms related to bulbar, limb, and respiratory function,[20] and the rate of change may predict survival.[21] However, metric analysis of the ALSFRS-R has suggested that it may not be an ideal measure of global function.[22] In addition, statistical handling of functional data after death is difficult.[23] Composite primary measures, such as the Combined Assessment of Function and Survival (CAFS), have been proposed.[24] The CAFS utilizes a unique approach, by ranking patients' clinical outcomes by combining survival time and change in the ALSFRS-R. Such composite endpoints may provide a more statistically robust measurement of clinical response than survival and functional data alone, and improve the likelihood of identifying a significant effect with treatment.

MUSCLE STRENGTH TESTING. Muscle strength may be quantified using composite manual muscle testing (MMT) scores, which usually involve averaging measures from multiple muscle groups using the Medical Research Council (MRC) muscle strength grading scale.[25] Additional quantitative methods have been used to evaluate muscle strength, including hand-held dynamometry (HHD) and custom measurement apparatus (see Table 2)[26,27]; HHD equipment in particular is inexpensive, and measurements may not be much more time-consuming than MMT. MMT, HHD, and other measures of muscle strength such as maximum voluntary isometric contraction (MVIC) demonstrate equivalent inter-rater reliability and reproducibility.[28,29]

Replacing MMT methods with more objective measurements of muscle strength such as HHD or MVIC in future studies may improve measurement for a number of reasons. For example, the MRC scale is non-linear, and is particularly insensitive at detecting changes in the range of strength measures covered by scores of 4 and 5 out of 5.[30] In contrast, both HHD and MVIC provide relatively linear measurements at different muscle strengths. MMT may be more sensitive to detect change than MVIC, likely due to greater numbers of muscles tested,[28] but this limitation of MVIC may be overcome by HHD. With appropriate training, objective muscle strength measurement apparatus may provide a more

TABLE 2. Candidate Biomarkers in ALS

Measurement	Advantages	Limitations	Recommended Strategies
Muscle strength			
MMT	No equipment barrier; rapid to perform; can measure a broad range of muscle groups	Nonlinear; insensitive to change in mild weakness categories	MMT remains useful for clinical monitoring, but more rigorous quantitative techniques are recommended for clinical research. HHD may be an ideal balance between equipment and time costs and accuracy.
MVIC	Linear; more sensitive to change than MMT for single muscle	Extensive equipment and training barriers to widespread application	
HHD	Minimal equipment requirements; rapid to perform; comparable accuracy to MVIC in weak muscles	Clear training effects; underestimates weakness above a force of 20kg	
Functional status			
ALSFRS-R	Clinically meaningful index; minimal training requirements; universal applicability	Statistical manipulation required to handle data after death; clinical heterogeneity distorts the link between total score and disease severity	ALSFRS-R provides useful guidance on patient progression. Composite measures may be better suited to trial design to reduce cost, duration, and patient recruitment burdens.
CAFS	Increases statistical power; improves statistical treatment of patient death; simultaneous analysis of 2 important endpoints (survival and function)	Clinically intangible	
Respiratory function			
VC	Widely available portable equipment; well-developed normative data	May not be reliable in patients with bulbar or facial weakness; affected by submaximal effort; may not be sensitive to detect mild to moderate respiratory muscle weakness; affected by chest wall and airway factors	SNIP balances ease of recording, reliability, and accuracy and hence may be the optimal approach.
MIP	Portable equipment; more sensitive to early respiratory weakness than FVC	May not be reliable in patients with bulbar or facial weakness;	
SNIP	Can be performed reliably in most ALS patients, including those with orofacial weakness; predicts respiratory failure more accurately than VC and MIP		

TABLE 2: Continued

Measurement	Advantages	Limitations	Recommended Strategies
Inspiratory esophageal pressure and trnsdiaphragmatic pressure	Most accurate measurement of respiratory muscle strength	Invasive procedure intolerable to some patients; equipment setup not available in all centers	
Surrogate markers of LMN loss			
Nerve conduction studies	Necessary operator experience and equipment widely available	Influenced by reinnervation changes and not a direct reflection of LMN loss; nonlinear	The ideal approach to quantify LMN loss has not been determined. MUNE has been extensively studied and is the most direct measure of LMN loss, but limitations have prevented its universal application. Consensus regarding the optimum MUNE technique, and simplification or automation of data acquisition and analysis will facilitate the widespread incorporation of MUNE into multicenter trials. Novel approaches including EIM and peripheral nerve diffusion tensor imaging may hold promise for future clinical studies.
MUNE	Direct measurement of LMN loss	Studies can be time consuming; training requirements are substantial	
Nerve excitability studies	Automated data recording; detailed physiological information regarding axonal function	Complex data analysis; necessary equipment and expertise presently limited to selected centers	
EIM	Easy to acquire recordings and analyze data; relatively rapid to perform; multiple muscle recordings; relatively linear change with progression	Measurements influenced by age and gender, subcutaneous fat distribution, and muscle changes from immobility; indirect measurement of LMN loss	
Muscle ultrasound	Quick and easy to perform; relatively low equipment needs and training requirements; changes detectable in clinically normal muscles	Wide variation in changes with progression; reproducibility of echogenicity measurements may be limited	
Surrogate markers of UMN loss			

TABLE 2: Continued

Measurement	Advantages	Limitations	Recommended Strategies
MRI techniques	Powerful measures of cortical atrophy and neuronal integrity (individual techniques detailed below); may detect and measure asymptomatic UMN involvement	Patients must lie flat in the scanner, which may be difficult if respiratory muscle weakness is present	In the absence of robust clinical UMN scales, a surrogate marker of UMN dysfunction may be considered critical in the design of future clinical trials. Primary motor cortex thickness and DTI of the rostral corticospinal tract may be ideal to provide structural information regarding UMN involvement, and with further development of the technique, TMS may provide important functional data.
MRI morphometry (VBM) and SBM	Synchronously evaluates multiple brain territories	Limited sensitivity to gray matter changes on a group level; inconsistent progression data from different longitudinal studies; images are normalized to standard templates, which may smooth out some data signal	
DTI	Useful to evaluate corticospinal tract integrity as well as other white matter tracts	Changes may not relate to clinical measures in some studies	
MRS	Noninvasive measurement of tissue metabolites	Inconsistent pattern of metabolite changes with disease progression; no standardized approach to analysis; low signal-to-noise ratio and resolution	
PET	Provides quantitative functional data; specific ligands may target individual neuronal pools	Exposure to ionizing radiation; requires facilities not available in all centers	
TMS	May detect UMN dysfunction in absence of clinical UMN signs; noninvasive; may be performed seated, hence tolerable in patients with respiratory insufficiency	Difficult to perform if severe hand muscle wasting is present; further longitudinal studies are needed	

ALS = amyotrophic lateral sclerosis; ALSFRS-R = revised ALS Functional Rating Scale; CAFS = Combined Assessment of Function and Survival; DTI = diffusion tensor imaging; EIM = electrical impedance myography; FVC = forced vital capacity; HHD = hand held dynamometry; LMN = lower motor neuron; MIP = maximal inspiratory pressure; MMT = manual muscle strength testing; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; MUNE = motor unit number estimation; MVIC = maximal voluntary isometric contraction; PET = positron emission tomography; SBM = surface-based morphometry; SNIP = sniff nasal inspiratory pressure; TMS = transcranial magnetic stimulation; UMN = upper motor neuron; VBM = voxel-based morphometry; VC = vital capacity.

universal means of assessing changes in muscle strength, remaining relatively independent of examiner and patient factors such as baseline muscle strength.

RESPIRATORY MUSCLE STRENGTH TESTING. Measurement of respiratory function has been included in most major ALS clinical trials, and may be easily performed in the clinic setting using portable spirometry units. Forced vital capacity (FVC) obtained at baseline may predict the rate of progression.[31] Maximal inspiratory pressure, sniff nasal inspiratory pressure (SNIP), and supine FVC may be more sensitive than routine seated FVC measurement in detecting respiratory insufficiency in ALS.[32,33] Reduction in slow vital capacity was found to be reduced in the treatment arm of the recently completed phase 2 trial of tirasemtiv.[34] FVC remains a routine measurement in the clinical care of patients with ALS but is flawed as a quantitative measurement of disease progression, particularly as it is often unreliable in patients with bulbar weakness, and may be insensitive to change in patients with mild to moderate respiratory muscle weakness. SNIP is recommended as a noninvasive measure of respiratory muscle weakness, as it can be performed reliably by most ALS patients, and is more sensitive to change in respiratory muscle strength than FVC.[35] Invasive techniques such as esophageal pressures are also accurate but impractical for regular use in the clinic.

QUANTIFYING UMN INVOLVEMENT. Identifying and quantifying UMN dysfunction has become increasingly important in the understanding and monitoring of ALS progression. However, clinical UMN abnormalities may be difficult to detect in limbs with significant LMN involvement, and pathological reflexes such as the Babinski sign may be unexpectedly absent in ALS patients.[36] Validated clinical UMN scores remain lacking, and imaging and neurophysiological techniques may hold greater promise as tools to quantify UMN dysfunction.

Candidate Biomarkers of Disease Progression

Clinical and functional measures alone may not be adequate indicators of the biological activity of the disease. Muscle reinnervation initially compensates for LMN loss (Fig 1), and substantial motor neuron degeneration may have already occurred prior to the development of clinical weakness,[37,38] making change in muscle strength or other clinical indices potentially insensitive to significant changes in the motor neuron pool. In addition, UMN degeneration is not readily quantified by clinical means.

A biomarker is a laboratory measurement intended as a substitute for survival endpoints or a clinically relevant functional outcome in therapy trials, and will ideally reflect the underlying biology of the disease. The FDA defines 4 categories of clinical biomarkers: diagnostic, prognostic, predictive, and pharmacodynamic.[39] A diagnostic biomarker is a disease characteristic that can be used to categorize patients. Prognostic biomarkers are baseline characteristics that inform about the natural history of the disease in the absence of therapy. A predictive biomarker is a disease characteristic that categorizes patients according to their likelihood of treatment response. Finally, pharmacodynamics biomarkers are measures that indicate a treatment effect.

An issue at present is that there are no validated biomarkers in ALS.[40,41] In ALS, the use of batteries of biomarkers to measure disease burden may provide more accurate and complete assessments of disease progression than clinical indices alone, and diagnostic, prognostic, predictive, and pharmacodynamics measures may all be relevant. Selected batteries would ideally reflect the complexity of motor system involvement in ALS. Existing and emerging markers of disease progression are discussed below, and strengths and limitations of each method are detailed (see Table 2).

Measures of LMN Loss

ELECTRODIAGNOSTIC STUDIES. Electrodiagnostic studies have an important role to play in the diagnosis of ALS, and may be useful to exclude mimic disorders such as multifocal motor neuropathy.[42] Disease progression in ALS is associated with progressive reduction of compound muscle action potentials (CMAPs) on motor nerve conduction studies (NCS).[43] Motor NCS parameters, specifically distal motor latency, CMAP amplitude, and F-wave frequency, may be used to derive the Neurophysiological Index,[44] which is sensitive to disease progression and may be appropriate as an outcome measure particularly in ALS clinical trials conducted over short time periods.[45] However, CMAP amplitude is also influenced by compensatory reinnervation, making it a suboptimal estimate of LMN loss.

MOTOR UNIT NUMBER ESTIMATION. Motor unit number estimation (MUNE) is a neurophysiological tool that aims to quantify residual motor axons supplying a muscle, by estimating the contribution of individual motor units to the maximal CMAP response (see Fig 1). A number of MUNE techniques have been developed, but there is as yet no consensus on the optimum methodology. Longitudinal studies of changes in MUNE in ALS have correlated loss of motor neurons with survival.[46]

The concept of motor unit number estimation was developed in 1971 by McComas et al,[47] who estimated MUNE as the ratio of the maximal CMAP divided by

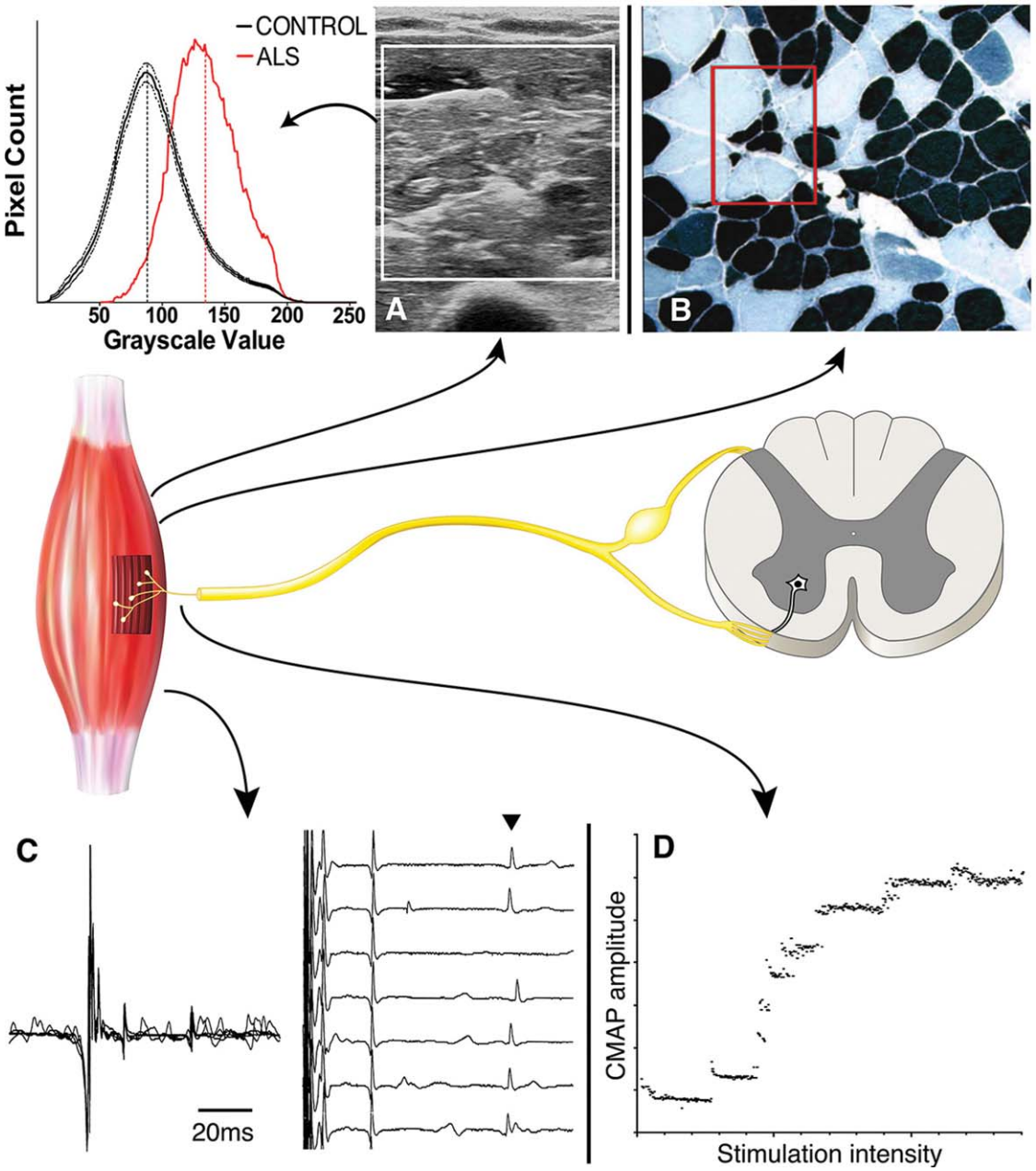

FIGURE 1: Markers of lower motor neuron loss. Illustration of the motor unit, comprising the anterior horn cell in the spinal cord projecting to innervate a group of muscle fibers. Methods used to measure loss of anterior horn cells are depicted. (A) Muscle ultrasound may show increased muscle echogenicity and reduced muscle thickness. A grayscale histogram derived from the depicted ultrasound image shows the distribution of grayscale values (red curve), superimposed onto average (± standard deviation) grayscale histograms of 44 normal control subjects (black curves). (B) Ultrasound changes reflect histopathological abnormalities with fiber-type grouping, suggesting reinnervation, and grouped atrophy (red box), suggesting motor neuron loss, typical of motor neuron diseases. (C) These muscle denervation and reinnervation changes may be identified on electromyography, with prolongation of individual motor units, as a result of dyssynchrony of muscle fiber firing secondary to poorly myelinated regenerating branches. Jitter and block of muscle fiber action potentials may be seen as a result *(arrowhead)*. (D) Anterior horn cell loss, independent of muscle reinnervation changes, may be quantified using motor unit number estimation techniques, in this instance using an incremental stimulation technique. ALS = amyotrophic lateral sclerosis; CMAP = compound muscle action potential.

the average single motor unit potential (SMUP). In this early work, incremental stimulation was used to determine the average SMUP; however, this technique may result in alternate or summative activation of units of similar thresholds and as such may overestimate motor unit numbers. To avoid this problem, the multiple point stimulation technique was developed, whereby SMUPs are collected by stimulating different points of the nerve with the resulting

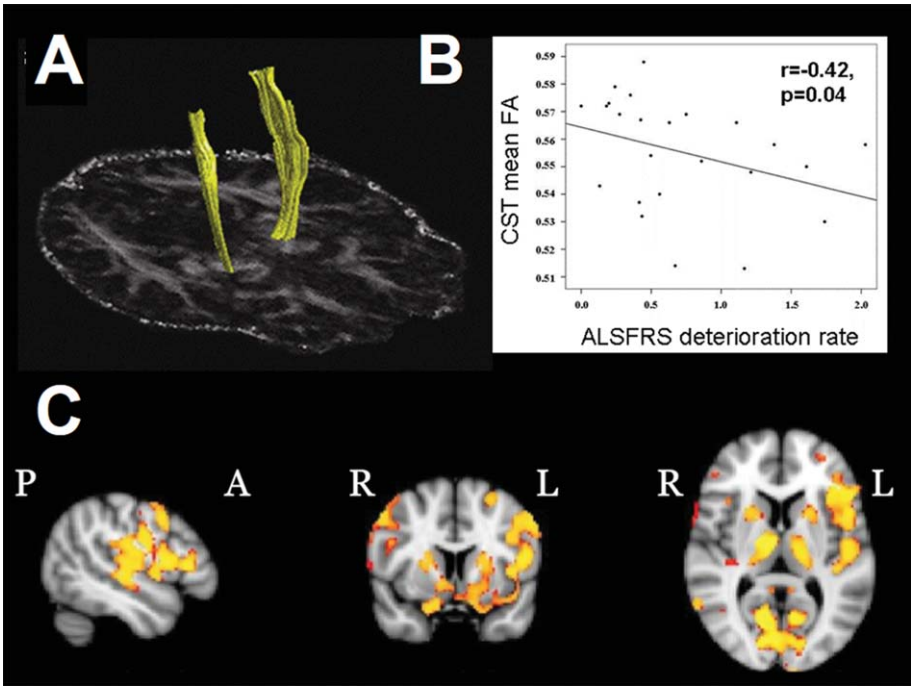

FIGURE 2: Brain imaging markers of disease. (A) The corticospinal tracts (CST) can be reconstructed using diffusion tensor tractography. (B) A scatterplot of the extracted mean CST fractional anisotropy (FA) against the rate of decline of Amyotrophic Lateral Sclerosis (ALS) Functional Rating Scale (ALSFRS) score (points per month) shows a negative correlation, with potential to prognostically stratify patients (adapted from Fig 3 in Turner et al[64]). (C) Longitudinal gray matter changes are extensive in ALS, detected using voxel-based morphometry. They include extramotor frontal lobe regions and basal ganglia (regions of significantly reduced gray matter density common to a large group of ALS patients over time, shown in yellow–red scale overlaid on standard brain image in 3 planes, with anterior [A], posterior [P], right [R], and left [L] marked).

average SMUP used to calculate MUNE.[48] An alternative technique, the statistical method, does not involve collecting individual SMUPs, but rather statistical handling of steps in amplitude on incremental stimulation.[49,50] Additional methods analyze the interference pattern of motor units recorded over the surface of the muscle.[51,52]

MUNE has shown good inter-rater and test–retest reliability[53] but does require substantial operator training. MUNE was incorporated as a secondary endpoint in a clinical trial of creatine in ALS.[54] In this trial, an intra-rater test–retest variability of up to 20% was accepted, which may be expected to blunt the sensitivity of MUNE to detect smaller treatment effects, and which compares unfavorably with variability in FVC measurements (5%), but is similar to the variability of maximal voluntary isometric contraction muscle strength measurements (up to 17%).[26] Newer nerve stimulation and analysis methods, such as multipoint incremental stimulation, motor unit number index, and Bayesian methods of statistical analysis, overcome a specific issue in ALS, which is variability of individual motor units with repeated stimulation, a result of conduction failure in immature regenerating nerve terminals from attempts at reinnervation, which may confound MUNE calculated using early statistical techniques.[55]

NERVE EXCITABILITY. Motor axonal dysfunction has been demonstrated in ALS patients using threshold-tracking nerve excitability studies, with increased persistent Na^+ conductance and reduced K^+ conductance identified.[56] Changes in axonal excitability evolve with disease progression,[57] and may be a predictor of survival in ALS patients.[58] Axonal excitability parameters may be useful biomarkers of axonal degeneration.

ELECTRICAL IMPEDANCE MYOGRAPHY. Electrical impedance myography (EIM)[59] is an emerging technology that relies on the strong directional dependence of current flows in muscle. EIM demonstrates good test–retest reliability, and changes in EIM measurements in ALS patients may be detected from muscles that are not yet clinically involved. Power calculations suggest that EIM may be superior to MUNE and manual muscle strength testing for the detection of deterioration in ALS,[60] and EIM shows promise as a biomarker for future clinical trials.

MUSCLE ULTRASOUND. Presently, the most established role of ultrasound in the ALS clinic relates to the identification of fasciculations.[61] Ultrasound may also detect changes in the thickness and echogenicity in muscles (see Fig 1) with and without clinical weakness,[61] which may provide supplementary evidence of muscle

TABLE 3. Potentially Quantifiable Cerebral Neuroimaging Markers in ALS

Quantifiable Neuroimaging Marker	Main Locations		Key References
	Cross-Sectional	Longitudinal	
MRI			
Gray matter density reduction (VBM)	PMC	PMC, frontotemporal cortex	105–107
Cortical thinning (SBM)	PMC	PMC, temporal cortex	108,109
Decreased fractional anisotropy, increased radial/mean diffusivity (DTI)	CST, CC, cervical cord	CST, CC, frontotemporal tracts, cervical cord	107,110–114
N-acetylaspartate (MRS)	PMC	PMC	11a, 116
PET			
Microglial activation (^{11}C-PK11195; ^{18}F-DPA-714)	PMC, thalamus, pons, DLPFC	—	117,118
Reduced $GABA_A$ receptor binding (^{11}C-flumazenil)	PMC, premotor	—	119
Reduced 5-HT_{1A} receptor binding (^{11}C-WAY100635)	Frontotemporal cortex	—	120

[a]-HT = 5-hydroxytryptamine; ALS = amyotrophic lateral sclerosis; CC = corpus callosum; CST = corticospinal tract; DLPFC = dorsolateral prefrontal cortex; DTI = diffusion tensor imaging; GABA = γ-aminobutyric acid; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; PMC = primary motor cortex; SBM = surface-based morphometry; VBM = voxel-based morphometry.

denervation. Muscle changes vary considerably with disease progression.[62] Muscle ultrasound is a relatively easy skill to acquire,[63] but variability of ultrasound measurements between different ultrasound systems, in particular muscle echogenicity, presently limits its applicability in multicenter studies.

Measures of UMN Dysfunction

IMAGING OF BRAIN AND SPINAL CORD. The clinical syndrome of ALS and its continuum partner frontotemporal dementia are, along with other neurodegenerations, emerging as systems-level, network-based cerebral disorders. Neuroimaging, led by magnetic resonance imaging (MRI), is poised to deliver biomarkers as part of a deeper understanding of brain structure and function. Routine clinical MRI for the exclusion of alternative pathology does not reveal reliable markers for ALS. Corticospinal tract T2-weighted hyperintensities have limited specificity (<70%) but lack sensitivity (<40%). However, advanced applications of MRI, and ligand-based positron emission tomography (PET) have generated several candidates with potential as a quantitative biomarkers of disease activity and progression (Table 3).[64]

Although motor symptoms are the hallmark of ALS, macroscopic atrophy of the motor cortex is typically confined to very rare cases of PLS. However, computerized MRI segmentation techniques have proved more sensitive in the whole brain assessment of cortical changes in ALS. Voxel-based morphometry detects regional gray matter density, and surface-based morphometry differences in a range of topographical measures across a reconstructed cortical ribbon. In broad terms, both techniques consistently demonstrate atrophy of the primary motor cortex in ALS, most strongly linked to clinical UMN involvement, and more variably to measures of disability. Evidence of frontotemporal cortical involvement has been less consistent in its location across studies, but temporal lobe cortical thinning has been linked to more rapid disease progression, in keeping with independent observations about cognitive involvement and prognosis.[15]

Diffusion tensor imaging (DTI) assesses the directional movement of water within the white matter, which is highly restricted (anisotropic) when confined within intact neuronal pathways, but able to move more freely in multiple directions (isotropic) where there are damaged tracts. DTI measures are quantifiable. The most consistent abnormalities in ALS are reduced fractional anisotropy and the related measures of increased radial and mean diffusivity, typically within the rostral corticospinal tracts and interhemispheric motor fibers of the corpus callosum, with a less consistently observed longitudinal change than cortical measures. Increasingly, there

appears to be merit in the extension of DTI to the spinal cord,[65] where there may be useful markers of LMN involvement in addition.[66]

Magnetic resonance spectroscopy allows the detection and quantification of tissue metabolites, typically within a small region of interest, but more recently using whole brain techniques. N-Acetylaspartate, a nonspecific marker of neuronal damage, is among the most easily identifiable metabolite peaks to quantify, and is consistently reduced in the primary motor cortex in ALS.

Finally, PET is a highly quantifiable technique, and specific receptor ligands, including those for microglial activation, and γ-aminobutyric acidergic and serotonergic systems have all demonstrated specific patterns of binding in ALS.

Structural MRI analysis relies on the normalization of the natural variations in brain size and shape to fit a predefined spatial template and allow standardized comparisons to be made at a group level. Such image transformations inevitably smooth away some of the potentially deeper phenotyping markers at the individual patient level. More focused multivariate region of interest analysis, larger control banks (perhaps based on disease mimics rather than healthy individuals), with standardization and harmonization of sequence acquisition and analysis, are all future steps on a roadmap to clinical translation.[67]

TRANSCRANIAL MAGNETIC STIMULATION. Transcranial magnetic stimulation (TMS) is able to improve the sensitivity of ALS diagnosis by demonstrating evidence of UMN dysfunction. Changes in cortical excitability may precede the development of muscle weakness in ALS.[68,69] TMS may also be a useful tool for monitoring the effect of therapy (eg, riluzole)[70] and the progression of UMN abnormalities in ALS, although further longitudinal studies are required to determine the nature of the changes over time. Hand muscles are frequently studied, and TMS becomes technically difficult if CMAP amplitudes fall below approximately 1mV. Hence, hand muscle atrophy with disease progression precludes longitudinal assessment with TMS in some patients. Like other techniques described here, there are equipment and training barriers to overcome.

FLUID BIOMARKERS. There has been vigorous interest in identifying biomarkers in biofluids of patients with ALS, such as cerebrospinal fluid (CSF), blood, and urine. Such biomarkers may serve as a means of distinguishing ALS from mimic disorders, for the purposes of prognostication, disease monitoring, and monitoring drug effects in treatment trials.

Protein-based biomarkers identified in ALS typically reflect neuronal loss or changes in inflammatory pathways. Neurofilament proteins may increase following axonal injury, and high levels have been identified in CSF and plasma of ALS patients.[71] Patients with more advanced disease show higher levels of antibodies against neurofilament proteins than those with earlier disease.[72] Serial neurofilament protein levels with disease progression reflect the speed of neurological decline and survival.[73] Conversely, TDP-43 decreases in the CSF with disease progression.[74] Concentrations of CSF glial activation markers correlate with survival time.[75]

Nonprotein biomarkers may also be of value. Serum creatinine represents a simple and inexpensive estimate of whole body muscle mass, although its concentration in the serum may be affected by renal dysfunction. More detailed metabolic signatures may be identified with proton nuclear magnetic resonance spectroscopy metabolomics of fluids such as CSF from ALS patients.[76]

Several small studies have explored panels of plasma and CSF biomarkers as a means to predict disease progression and to distinguish ALS patients from controls.[77–79] These studies have identified inflammatory cytokines, growth factors, and markers of iron metabolism as possible markers of disease. Although the link between these markers and disease pathogenesis is unclear, further exploration of CSF and plasma biomarker panels in larger ALS studies may provide important prognostic biomarkers and measures to evaluate treatment response.

Implications for Clinical Trial Design

More detailed understanding of genetic and pathophysiological mechanisms in ALS has drawn advances in symptomatic and disease-modifying therapy to the horizon. However, with this expansion of opportunity there comes a considerable need to rationalize the process of therapy development.

Accurate phenotypic classification and balancing treatment groups for different phenotypic subtypes may prevent the skewing of disease progression data in clinical trials due to expected variation in the natural history. Clinical trials have separated patients into bulbar and spinal subtypes, defined by the region of onset, in an attempt to balance the phenotypic representation between treatment groups. However, methods of phenotypic classification clearly need revision. For example, 1 study identified 5 phenotypic clusters, 1 of which had no deaths, 1 with a median survival of 14 years, and another with a median survival of 8 years.[80] As such, simply dividing patients into bulbar and spinal onset may neglect substantial phenotypic variation within those subgroups.

There is clearly a need to better define ALS populations beyond clinical classification, and this will require a greater effort to identify and validate disease-relevant biomarkers. Such biomarkers must also be applied in the appropriate clinical trial context. For example, whereas repeated neuroimaging for quantification of fractional anisotropy in a large multicenter phase 3 study may be impracticable, such a measurement could be exceedingly important in establishing efficacy in a smaller phase 2 study. This concept of "fit for purpose" is important when considering the optimum approach to clinical trial design.

Comprehensive characterization of patients entered into clinical trials including genetic delineation will be critically important to facilitate widespread clinical application of drug discovery efforts through efficient clinical trial design. International collaborative efforts and the mandatory integration of biomarker components to all future therapeutic trials will inevitably advance these aims.[81]

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Authorship

N.G.S.: study design, literature review, drafting and revising the manuscript, preparation of figures. M.R.T.: drafting the neuroimaging section, critical revision of the manuscript for important intellectual content, preparation of figures. S.V.: critical revision of the manuscript for important intellectual content, preparation of figures. A.A.-C.: drafting the genetics section, critical revision of the manuscript for important intellectual content. J.S.: critical revision of the manuscript for important intellectual content. C.L.-H.: drafting the motor unit number estimation section, critical revision of the manuscript for important intellectual content. M.C.K.: study supervision, critical revision of the manuscript for important intellectual content.

Potential Conflicts of Interest

S.V.: consultancy, Biogen, Bayer Schering. J.S.: board membership, Biogen advisory board; consultancy, Cytokinetics, GlaxoSmithKline; grants/grants pending, ALS Association, Muscular Dystrophy Association, NIH.

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