

## Advances in molecular pathology, diagnosis, and treatment of amyotrophic lateral sclerosis

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### Abstract

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Although the past two decades have produced exciting discoveries in the genetics and pathology of amyotrophic lateral sclerosis (ALS), progress in developing an effective therapy remains slow. This review summarizes the critical discoveries and outlines the advances in disease characterization, diagnosis, imaging, and biomarkers, along with the current status of approaches to ALS care and treatment. Additional knowledge of the factors driving disease progression and heterogeneity will hopefully soon transform the care for patients with ALS into an individualized, multi-prong approach able to prevent disease progression sufficiently to allow for a dignified life with limited disability.

### Introduction

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As the most common cause of adult onset motor neuron disease (MND), amyotrophic lateral sclerosis (ALS) is traditionally classified as a neuromuscular disorder because the presenting symptoms are caused by muscle weakness and atrophy. However, clinical, genetic, and molecular discoveries over the past 20 years have challenged this convention. ALS shares features of fron-

frontotemporal dementia, a group of neurodegenerative disorders that causes cognitive, behavioral, and motor dysfunction. Nearly half of all patients with ALS have varying degrees of cognitive and/or behavioral impairment, with approximately 15% meeting the diagnostic criteria for frontotemporal dementia.<sup>1</sup> Conversely, about 15% of patients with behavioral variant frontotemporal dementia and 18% of patients with primary progressive aphasia have ALS.<sup>2 3</sup> These disorders also have overlapping genetics, with hexanucleotide repeat expansion (HRE) in *C9ORF72* being the most common genetic cause of ALS, frontotemporal dementia, or both in people of European ancestry.<sup>4</sup> Additionally, abnormal aggregation of transactive response DNA binding protein 43 (TDP-43) or fused in sarcoma (FUS) is present in the cytoplasm of cortical neurons in ALS and frontotemporal dementia.<sup>5 6</sup> As such, ALS is widely recognized as a complex neurodegenerative disorder in the frontotemporal dementia-MND continuum.<sup>7 8</sup> The reconceptualization of ALS in this continuum of disorders has allowed for novel approaches toward understanding fundamental disease mechanisms contributing to pathogenesis and has opened new avenues in approaches toward therapy. In this review, we provide a comprehensive summary of the clinical and genetic heterogeneity of ALS and advances in molecular pathology and biomarkers, and we highlight key interventions that improve quality of life. The intended audience includes students, trainees, general neurologists, and neuromuscular subspecialists.

## Epidemiology

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The worldwide prevalence and incidence of ALS are estimated to be 4.42 per 100 000 population and 1.59 per 100 000 person years, respectively, and population based studies have shown geographic variation with the highest in western Europe (prevalence 9.62 per 100 000 population and incidence 2.76 per 100 000 person years) and lowest in South Asia (prevalence 1.57 per 100 000 population and incidence 0.42 per 100 000 person years). The incidence and prevalence of ALS are higher in developed regions, and a temporal trend has been observed, with the incidence rising by 0.00013 per year.<sup>9 10</sup> The prevalence and incidence of ALS is higher in men (prevalence 5.96 per 100 000 population; incidence 1.91 per 100 000 person years) than in women (prevalence 3.90 per 100 000 population; incidence 1.36 per 100 000 person years).<sup>9 10</sup>

## Sources and selection criteria

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We independently did searches using the Boolean search criteria in the PubMed and Embase databases, between January 1990 and December 2022, using search terms such as amyotrophic lateral sclerosis, motor neuron disease, frontotemporal dementia, diagnosis, diagnostic criteria, prognosis, genetics, pathology, biomarker, and treatment. We identified articles published in the English language and selected them for inclusion on the basis of other criteria including relevance, peer review, and study type (randomized controlled trials, systematic reviews and meta-analyses, and observational studies). We prioritized publications in high impact and ALS specific journals published in the past 15 years. Several important publications could not be included owing to the scope of this review. We excluded case reports and articles not published in English.

## Clinical complexity of ALS

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### Clinical heterogeneity

ALS is a clinically heterogeneous disorder ([fig 1](#)), and the biological underpinning of the heterogeneity is poorly understood. Typically, the symptom onset is localized with spread of motor impairment to adjacent muscle groups and/or regions of the neuroaxis. Usually, the progression rate is linear for any given person, but the rate often varies between patients.<sup>11</sup> ALS of spinal onset with weakness first appearing in limb muscles occurs most frequently (two thirds of patients), followed by bulbar onset with initial weakness in lingual and oropharyngeal muscles (a third of patients). Axial or respiratory muscles are rarely the first to be affected.<sup>12</sup> Uncommon subtypes of ALS with spinal or limb onset exist, with atypical patterns of weakness in which the motor impairment tends to be regionally confined early in the disease course. In brachial amyotrophic diplegia or flail arm syndrome, weakness tends to affect proximal upper extremities symmetrically. Similarly, in flail leg syndrome or lower extremity amyotrophic diplegia, weakness is mainly in the lower extremities.<sup>13</sup> Other rarer ALS phenotypic variants include isolated bulbar ALS and hemiplegic ALS presenting with asymmetric hemibody weakness.<sup>14</sup> Although the symptoms rarely remain restricted in these subtypes, the progression is typically slow.<sup>12</sup>

Another contributor to phenotypic heterogeneity is the burden of neuronal degeneration in the cortex (upper motor neuron; UMN), brainstem, and spinal cord (lower motor neuron; LMN). Typically, the neurological examination shows UMN and LMN dysfunction, but the contribution of each likely falls on a continuum and can vary with patients having predominantly upper or lower motor signs ([fig 1](#)).<sup>11</sup> One explanation for this variability may be differences in the pattern of spread in the course of the disease.<sup>11 15</sup> At the extremes are rare phenotypes such as primary lateral sclerosis (PLS) presenting as a pure UMN disorder and progressive muscular atrophy (PMA) presenting as a pure LMN disorder.<sup>16 17 18</sup> Some clinical features distinguish PLS from typical ALS. In PLS, symptoms are symmetric and slowly progressive, and they frequently have an ascending pattern of spread.<sup>19</sup> PMA is clinically similar to typical ALS in the rate and pattern of symptom spread, but a subgroup of PMA may have slower disease progression.<sup>17</sup> Prognostic factors associated with longer survival include UMN or LMN predominant symptoms, flail arm variant, and younger age at onset. Factors associated with shorter survival include bulbar and/or respiratory onset, comorbid frontotemporal dementia, poor nutritional status, neck flexion weakness, and older age at onset.<sup>12</sup>

### Cognitive and behavioral dysfunction

Although ALS is synonymous with MND, cognitive and/or behavioral dysfunction are recognized core clinical features.<sup>1</sup> Neuropsychological abnormality is associated with faster disease progression and shorter survival and occurs more frequently in advanced disease.<sup>20</sup> Motor symptoms that alert patients and their care givers to a neurological disorder may overshadow antecedent or concurrent neuropsychological symptoms.<sup>21</sup> Because cognitive or behavioral changes may be obscured by motor dysfunction, validated screening tests specific for ALS such as the Edinburgh Cognitive and Behavioral ALS Screen or ALS Cognitive Behavioral Screen are recommended in all

patients.<sup>22 23</sup> If the screening test is abnormal, a more extensive neuropsychological evaluation can determine the cognitive and/or behavioral changes.<sup>24</sup> ALS specific behavioral measures such as the Motor Neuron Disease Behavior Scale, the ALS-FTD-Questionnaire, or the Frontal Behavioral Inventory-ALS Version can be used to characterize and assess the severity of behavioral dysfunction.<sup>25 26 27</sup> The findings of these tests can classify patients as having ALS with cognitive impairment, ALS with behavioral impairment, ALS with combined cognitive and behavioral impairment, ALS with frontotemporal dementia (ALS-FTD) ([fig 2](#)),<sup>24</sup> or none of the above (no cognitive or behavioral impairment). Approximately half of all patients with ALS will show impairment on a comprehensive assessment, with approximately 5% classified as ALS with combined cognitive and behavioral impairment, 8% as ALS with behavioral impairment, 17% as ALS with cognitive impairment, and 15-20% as ALS-FTD.<sup>1 28 29 30</sup>

The most commonly affected cognitive domain in ALS is executive function, with abnormal verbal fluency being a consistent and sensitive marker even after control for bulbar motor dysfunction.<sup>31 32 33</sup> In patients who develop cognitive symptoms, impaired word fluency is an early finding.<sup>33</sup> Other features of executive dysfunction such as mental inflexibility, inattention and disinhibition, or inability to plan or problem solve can emerge as the disease progresses.<sup>1 20 22</sup> Impairment in multiple cognitive domains is less common in ALS and, when present, tends to involve language or memory and may be confounded by co-pathology such as Alzheimer's disease.<sup>1 34</sup> Isolated amnesic syndrome is not a feature in ALS and should prompt evaluation for an alternative cause. Overall, progression of cognitive dysfunction in ALS is slow and may remain stable over time.<sup>33</sup>

Behavioral abnormalities are a frequent neuropsychiatric feature in ALS, apathy being the most common. Others include disinhibition, perseverative behavior, change in food preferences, loss of empathy, or impaired social cognition including emotional processing.<sup>35 36 37 38 39</sup> Pathological crying and laughing, also known as emotional lability or pseudobulbar affect, is present in approximately one in three patients with ALS and is associated with gray and white matter pathology in the cortico-cerebellar network.<sup>40 41</sup> Pathological crying and laughing does not correlate with neuropsychological measures and should be distinguished from other cognitive and behavioral symptoms.<sup>42</sup>

### Diagnostic criteria and disease progression measures

ALS is a clinical diagnosis requiring findings of progressive motor neuron dysfunction in the absence of an alternative diagnosis. In typical ALS, few tests are needed to support the diagnosis and exclude mimics because other disorders rarely mimic ALS perfectly. The most common tests obtained in the diagnostic process are electrophysiology to establish a lower motor neuronopathy and neuroimaging of the brain and spine to exclude mimics causing structural abnormalities as a cause for UMN dysfunction. Routine laboratory studies are frequently obtained to exclude other causes of a patient's symptoms and are typically normal in ALS. The first widely used criteria in ALS were the El Escorial criteria, which aimed to provide a standardized diagnostic framework to conduct clinical research, with subsequent revisions and updates improving sensitivity allowing for earlier enrollment in clinical trials.<sup>43 44</sup> Although El Escorial and Awaji criteria are useful in clinical research, they are hampered by the heterogeneity of ALS and do not capture the full disease spectrum. For example, cognitive impairment and behavioral impairment are not included in

these criteria and pure lower motor neuron variants are excluded.<sup>43</sup> Furthermore, patients with ALS do not necessarily progress through the El Escorial categories of diagnostic certainty and may never attain the criteria for clinically definite ALS.<sup>45</sup> The ALS-frontotemporal spectrum diagnostic (ALS-FTSD) criteria proposed by Strong and colleagues use three diagnostic axes to define MND, cognitive and behavioral dysfunction, and other non-motor features.<sup>24</sup> Although the ALS-FTSD criteria more fully incorporate the ALS-FTD spectrum disorders, they still rely on El Escorial and Awaji criteria to define MND. The recently proposed Gold Coast criteria have attempted to simplify the diagnosis and recognize the potential utility of the development of biomarkers; however, further validation in different populations will be needed before routine use in clinical care or research.<sup>46</sup>

Variability in rate of symptom progression and survival in ALS represents a major obstacle in clinical trials.<sup>47</sup> Disease progression, most often measured by the decline in the revised ALS functional rating scale (ALSFRS-R) over time, differs considerably between patients, and survival ranges from less than one year to more than 10 years.<sup>47 48</sup> Respiratory failure is the most common cause of death. Clinical factors that contribute to heterogeneity in survival include age at symptom onset, sex, site of symptom onset (bulbar versus spinal), time to diagnosis, respiratory measures, pre-symptomatic body mass index, cigarette use, genetics, and the diagnosis of FTD.<sup>49 50 51</sup> Neurofilament, a biomarker for neurodegeneration, is a predictor of the progression and prognosis of ALS.<sup>52 53</sup> A recently proposed survival prediction model for ALS identified eight prognostic predictors and generated five different survival groups applicable to European patients at the individual level.<sup>54</sup> This model is an important step toward more effective stratification of patients in clinical studies, but validation in non-European groups is needed, and the model will likely evolve as other predictors are identified.

Clinical staging is an important tool for research and care planning because it informs the extent and severity of disease. The two proposed staging systems are the King's staging system and the Milano-Torino staging systems.<sup>55 56</sup> The King's staging system is defined by the number of body regions affected and bulbar and respiratory failure, whereas the Milano-Torino system uses the number of impaired domains as delineated by the ALSFRS-R to define successive stages.<sup>57 58</sup> These systems provide parallel clinical information, using different measures to establish escalating stages, and both have been used to analyze patient population data and are promising endpoints for clinical trials.<sup>58 59 60</sup> A limitation of both systems is the lack of cognitive and behavioral change captured by staging, although higher disease stage portends more severe cognitive impairment.<sup>29</sup>

## Advances in molecular biology

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### Molecular pathology

A new chapter in ALS pathology began in 2006 with the discovery of TDP-43 as the major constituent of ubiquitinated aggregates in motor neurons of sporadic ALS and most familial ALS and in cortical neurons in a subgroup of frontotemporal dementia.<sup>5</sup> TDP-43 staining is routinely done in postmortem tissue to characterize the pathology when ALS is suspected.<sup>61</sup> Abnormal accumulation of the protein as either neuronal or glial cytoplasmic inclusions or aggregates is found in 97%



of cases of sporadic ALS. Rarely, TDP-43 pathology is not a feature and is seen in ALS caused by superoxidase dismutase 1 (*SOD1*) or fused in sarcoma (*FUS*) gene mutations. Although accumulation of wild type TDP-43 has become the pathological hallmark of ALS, mutations in *TDP-43* are rare and are found in 4-5% of dominantly inherited familial ALS and 1% of sporadic ALS. Additionally, cytoplasmic TDP-43 aggregation can be seen in Alzheimer's disease, atypical parkinsonism, dementia with Lewy bodies, and limbic predominant age related TDP-43 encephalopathy, leading to the recognition of this group of neurodegenerative disorders as TDP-43 proteinopathies.<sup>62 63</sup> TDP-43 was first discovered in 1995, and its function was described as a suppressor of HIV-1 expression. As an RNA/DNA binding protein, it is involved in multiple processes such as RNA processing and maturation, RNA transport, microRNA maturation, and stress granule formation. It normally shuttles between the nucleus and the cytoplasm.<sup>63</sup> The cellular dysfunction leading to TDP-43 aggregation in the cytoplasm and the resultant neurodegeneration is a topic of active research.<sup>64 65</sup> Both loss-of-function and gain-of-function mechanisms have been proposed. An example of the loss-of-function mechanism is the TDP-43 function as a repressor of cryptic exon inclusion.<sup>66</sup> As a result of depletion from the nucleus and loss of the repressor function, cryptic exons (exons that are otherwise excluded from the mRNA) are included in at least two known loci, *STMN2* and *UNC13A*, causing reduced protein expression.<sup>67 68 69 70</sup> Of note, cryptic exons are not always shared between species, necessitating the development of new, humanized models. The mild motor neuron degeneration and the inability to replicate the loss of nuclear localization with concomitant cytoplasmic accumulation in animal models have been considered as evidence that these may be late events in the pathogenic cascade.

## Genetics

Whereas the vast majority of ALS is classified as sporadic disease—that is, without known history of another family member with either ALS or frontotemporal dementia—approximately 10% is familial ALS, which can be autosomal dominant, autosomal recessive, or X linked. The list of different causative genes for ALS has grown tremendously (>40 genes), mostly owing to advances in sequencing technologies. Genes causing two thirds of familial ALS and 10% of sporadic ALS are known. Only an overview of the genetics of ALS will be provided here ([table 1](#)), as detailed descriptions of all known ALS associated genes are beyond the scope of this review and can be found in other publications.<sup>71</sup>

**SOD1** The earliest understanding of the pathobiology of ALS derived from disease models based on mutations in *SOD1*, the first gene discovered in familial ALS in 1993, which accounts for 20% of familial ALS.<sup>72</sup> Several different mechanisms of neurodegeneration have been proposed, including conformational instability of *SOD1* protein, interactions with other proteins, and formation of toxic aggregates, but the exact mechanism remains unclear.<sup>73 74</sup> The consensus is that the many mutations spanning the whole length of *SOD1* confer a toxic gain of function, which has led to development of silencing mutant gene expression as a therapeutic approach.<sup>75 76</sup>

**C9ORF72** The largest genetic contributor to familial ALS was not discovered until 2011 because it is an HRE in an intron of a previously unknown gene named after the region of the chromosome where it is located, *C9ORF72* (chromosome 9, open reading frame 72). Repeats are typically not detectable by standard sequencing methods and require instead repeat primed polymerase chain

reaction (PCR), a PCR that overcomes the limitations of standard PCR by flanking the repeat region,<sup>77</sup> or a careful analysis of the reason for a drop-off in the sequence coverage of the region in standard next generation sequencing.<sup>4</sup> Intronic nucleotide repeat expansions are known to occur in genes linked to different disorders including myotonic dystrophy, spinocerebellar ataxias (SCA10, SCA 31, SCA 36), and Friedreich's ataxia. In unaffected people, the *C9ORF72* alleles have approximately two to 25 repeats. In contrast, ALS and/or frontotemporal dementia linked to chromosome 9 carry one normal allele and one expanded allele that can have hundreds to thousands of repeats. Somatic instability may further complicate the assessment of the repeat size, as different tissues may have different repeat sizes even within a single patient. The process by which HRE lead to neurodegeneration is not precisely known; however, three major hypotheses have been proposed: the (G<sub>4</sub>C<sub>2</sub>) repeats function similarly to repeat expansions in other disorders (myotonic dystrophy, fragile X tremor/ataxia syndrome) binding and sequestering RNA binding proteins impairing their ability to regulate RNA targets; HRE may cause epigenetic changes resulting in decreased *C9ORF72* mRNA expression; and an atypical mode of polypeptide translation across expanded repeats despite absence of an initiating codon, known as repeat associated non-ATG translation, which is also seen in spinocerebellar ataxia 8 and myotonic dystrophy.<sup>78 79</sup>

**Epigenetics** Similar to the growing list of familial ALS linked genes, our knowledge of genetic modifiers in sporadic ALS has increased. Expression levels of genes such as *EphA4*, which encodes a tyrosine kinase receptor that regulates developmental axon outgrowth, inversely correlate with age of disease onset and survival.<sup>80</sup> Variants of *ANG* increase the risk of development of ALS and Parkinson's disease,<sup>81 82</sup> and variants in *NEK-1* (NIMA (never in mitosis gene-A) related kinase-1) were found in nearly 3% of ALS patients.<sup>83</sup> An intermediate length ataxin 2 gene (*ATXN2*) polyglutamine repeats (>23 but <34 polyglutamine repeats) was found in some patients with sporadic ALS, and this finding was later confirmed in additional cohorts.<sup>84 85 86</sup>

Although interactions between polymorphisms and causative genes of ALS were previously appreciated, the idea that some families with ALS can harbor more than one of these genes was new. Van Blitterwijk et al discovered mutations in more than one ALS linked gene in five out of 97 families.<sup>87</sup> As our knowledge grows, we are likely to find more complex genetic interplay as the basis for disease in individual families.<sup>88</sup> These genes contribute to less than 1% of familial ALS, but their discovery has identified three main cellular functions that, when abnormal, can lead to neuronal degeneration in ALS: RNA/DNA metabolism, protein turnover/autophagy, and cytoskeletal and vesicular regulation ([fig 2](#)). This will hopefully improve understanding of disease mechanisms in the search for treatable targets.

## Advances in biomarkers

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### Imaging

Neuroimaging is used to look for structural abnormalities in the central nervous system that can mimic symptoms and signs of ALS. In most patients with ALS, the brain and spinal cord appear unremarkable or show non-specific abnormalities including corticospinal tract hyperintensity or motor cortex hypointensity in T2 weighted magnetic resonance imaging (MRI) sequences ([fig 3](#)).<sup>89 90</sup> Advanced imaging methods are powerful non-invasive research tools that can be used to study

and quantify structural, functional, and metabolic abnormalities.<sup>91</sup> For example, voxel based morphometry and surface based morphometry can determine global or regional gray matter atrophy and cortical thinning, and diffusion tensor imaging (DTI) can evaluate the integrity of white matter tracts.<sup>89</sup> Task based and resting state functional MRI can identify differing patterns of blood oxygen level dependent (BOLD) activity to interrogate connectivity neural networks, and magnetic resonance spectroscopy (MRS) allows for quantification of neuronal and glial metabolites such as N-acetylaspartate, a marker of neuronal integrity, creatine, a marker of energy metabolism, choline, a marker of cell membrane, and myo-inositol, a glial marker.<sup>92 93</sup> Other imaging modalities such as positron emission tomography (PET) can show regional changes in brain metabolism by using different receptor ligands.<sup>94</sup> The use of these tools has limitations in research, but technical improvements may prove them useful for group stratification in clinical trials, tracking disease progression, and predicting disease onset in pre-symptomatic carriers of gene mutations. Additionally, they have the potential to provide greater insight into the evolution of pathology.

The most frequent abnormal findings in structural brain imaging studies in ALS are thinning of the motor cortex and atrophy of the precentral gyrus (fig 3) and structural integrity loss in the corticospinal tract and the corpus callosum.<sup>15 95 96 97 98 99 100</sup> Morphometric changes correlate with the clinical phenotypes and the site of symptom onset, supporting the hypothesis of focal disease onset.<sup>100 101 102</sup> Another clinical-imaging correlation is the association of cognitive and/or behavioral impairment with extra-motor gray matter volume loss and white matter DTI diffusivity changes.<sup>103 104 105 106 107</sup> The structural abnormalities, however, do not consistently correlate with measures of disease progression such as the ALSFRS-R.<sup>101 103 108 109 110 111 112</sup> More widespread frontal atrophy is associated with faster disease progression and is consistent with the observation that faster disease progression occurs in patients with cognitive and behavioral impairment.<sup>113 114</sup> Longitudinal imaging is invaluable to track disease progression; however, studies show conflicting findings, with a few studies showing progressive gray and white matter changes over time, whereas others show no discernible changes.<sup>97 100 102 109 111 115</sup> The causes of these differences include unequal or small sample sizes, clinical heterogeneity, variable follow-up intervals, and different data acquisition and analysis methods. The role of neuroimaging to track clinical progression in ALS remains unresolved, but evolving imaging changes may mirror spread of pathology (for example, TDP-43).<sup>116 117 118 119</sup>

In ALS, MRS typically shows decreases in N-acetylaspartate or in N-acetylaspartate/creatine or N-acetylaspartate/choline in the motor cortex and brain stem corresponding to neuronal degeneration.<sup>120 121 122 123</sup> MRS indices correlate with clinical UMN disease burden in some studies, but their association with functional and cognitive measures are less consistent.<sup>120 121 122 124</sup> Additionally, longitudinal MRS studies are often limited by small sample size.<sup>121 125 126</sup> Other metabolites such as  $\gamma$ -aminobutyric acid and glutamate have been examined but need further validation.<sup>122 127</sup>

Early PET studies in ALS using the ligand <sup>18</sup>fluorodeoxyglucose show diffusely reduced uptake in the cortex and deep gray nuclei, mostly in patients who have signs of UMN dysfunction.<sup>128</sup> Other studies show hypometabolism in the frontal regions and hypermetabolism in the temporal regions, cerebellum, and upper brainstem.<sup>129 130 131</sup> PET ligands binding to the dopamine D2/D3, 5-hydroxytryptamine 1A, and  $\gamma$ -aminobutyric acid A receptors have been examined in ALS, suggest-



ing widespread neuronal dysfunction or degeneration.<sup>132 133 134</sup> More recently, interest has been growing in examining the role of neuroinflammation in ALS. This has led to the development of PET ligands that bind to the 18-kDa translocator protein expressed by activated glial cells. Studies using this ligand show increased uptake in the primary motor cortex and frontal regions that also show structural and metabolic abnormalities.<sup>135 136 137</sup> Additional studies are needed to understand the complex interactions between neuronal and glial cells in ALS.

In ALS, task based functional MRI shows increased activation of contralateral, and sometimes ipsilateral, brain regions such as the supplementary motor areas, sensorimotor cortex, temporal regions, deep gray nuclei, and cerebellum.<sup>138 139 140 141</sup> These abnormalities are hypothesized to represent adaptive or compensatory responses to the neurodegenerative process. Unlike task based functional MRI, resting state functional MRI shows varying patterns of coherence in the spontaneous BOLD activity. In ALS, the functional connectivity of brain regions can increase, decrease, or be mixed within different brain networks.<sup>15 142 143</sup> The variability in findings can be attributed to methodological differences. Regional decrease in functional connectivity in default mode and sensorimotor networks correlate with greater functional impairment.<sup>143 144</sup>

## Electrophysiology

**MUNE, MScanFIT, MUNIX** Motor unit number estimation (MUNE) is a promising electrophysiological technique to track disease progression in ALS by estimating the number of motor units in a muscle. Distal small muscles such as the intrinsic hand muscles (that is, abductor pollicis brevis) are examined. The concept of MUNE extends from the observation that incremental increases in the intensity of a stimulus delivered to the motor nerve results in stepwise increases in the amplitude of the compound muscle action potential (CMAP) recorded at the innervated muscle. If the average size of a single motor unit potential contributing to the CMAP can be determined, an estimate of the motor units in that nerve can be calculated by dividing the maximal CMAP amplitude by the average single motor unit potential amplitude.<sup>145</sup> Different methods to determine the average amplitude to calculate MUNE have been developed on the basis of this principle and applied in clinical studies in ALS.<sup>146</sup> Across studies using different methods, MUNE declines with disease progression and correlates with functional rating scales.<sup>147 148 149</sup>

More recently, the MScanFit MUNE method was developed to estimate the number of motor units from an objective stimulus response curve.<sup>150</sup> MScanFit MUNE may be more accurate, reliable, and easier and quicker to perform and may detect earlier motor neuron loss than other MUNE methods.<sup>151</sup> One limitation is the accessibility of nerves to peripheral stimulation, precluding its use to assess larger proximal muscles. The ease of applying this technique and ability to perform the study using standard electromyography machines make MUNE an attractive biomarker.

Motor unit number index (MUNIX) is an electrophysiological method that estimates the number and size of motor units by recording the maximum CMAP and epochs of surface electromyographic interference pattern at varying force levels.<sup>152</sup> MUNIX can be easily used to assess proximal and distal muscles and has been shown to track disease progression in ALS clinical trials.<sup>153</sup>

MUNIX values also decline before development of muscle weakness and may be more sensitive to detect early motor neuron loss.<sup>154 155</sup> However, the inter-rater variability across sites may limit the use of this method in clinical trials.<sup>154</sup>

**Transcranial magnetic stimulation** Transcranial magnetic stimulation (TMS) is a non-invasive electrophysiological technique that can objectively assess the integrity of the corticospinal motor neurons. Several different parameters can be measured, including motor threshold, motor evoked amplitude, central motor conduction time, cortical silent period, and intracortical facilitation or inhibition.<sup>156</sup> Compared with controls, the motor threshold is decreased and the motor evoked amplitude is increased in early ALS.<sup>156 157</sup> Other TMS findings include reduced duration of the cortical silent period with increasing stimulation intensity, reduced short intracortical inhibition, and increased intracortical facilitation.<sup>157 158</sup> Collectively, these abnormalities reflect altered cortical excitability. The resting threshold and central motor conduction time have been shown to correlate with clinical findings of UMN dysfunction and are suggested to be useful for tracking disease progression.<sup>126 159</sup> Further studies are needed to establish TMS as a robust biomarker of disease progression.

**Electrical impedance myography** Electrical impedance electromyography (EIM) is mostly a research tool to assess the health of the muscle. It is based on recording the voltage that results from applying a weak, high frequency electrical current across sampled muscle without inducing myofiber or neuronal action potentials. The volume conduction properties of the muscle depend on how strongly muscle resists or conducts alternating electrical current (conductivity) and on its ability to store electrical charge within (relative permittivity). These properties have been shown to differ in health and disease in murine models and patients.<sup>160 161</sup> EIM was first used to evaluate the muscle in Duchenne muscular dystrophy, and has more recently been used in ALS.<sup>161</sup> EIM parameters correlate moderately with standard ALS disease progression measures and MUNE. Surface EIM can be done at home, requires minimal training, is painless and repeatable, and has been assessed as an exploratory endpoint in different clinical trials. A motivated patient with ALS can collect surface EIM data more frequently than is done in standard clinical trials, thereby reducing the number of patients needed for a study.<sup>162</sup> An in-depth discussion of the advantages and limitations of both surface and needle EIM can be found in a recent review and subsequent letters to the editor.<sup>163 164 165</sup> Overall, EIM is a tool in development that may aid ALS patients and researchers to track disease progression.

## Fluid based biomarkers

Significant efforts are being made to evaluate and validate ALS biomarkers of various types (diagnostic, prognostic, predictive, and pharmacodynamic).<sup>166</sup> Neurofilament light chain (NFL) and phosphorylated neurofilament heavy chain have been examined in cerebrospinal fluid and serum in patients with ALS as a marker of neuronal injury.<sup>167 168 169</sup> Although concentrations are lower in serum than in cerebrospinal fluid, serum is more accessible and can be measured reliably using technologically advanced methods such as single molecule array technology (simoa).<sup>170</sup> In simoa, single molecules are trapped individually in wells followed by a digital readout of beads that are bound to their targets, leading to increased sensitivity for detecting protein at subfemtomolar concentration. Higher neurofilament concentrations tend to correspond to faster disease progres-

sion, a feature that can be explored in the design of future clinical trials.<sup>171</sup> A study of pheno-converters (pre-symptomatic carriers of causative genes for ALS who develop symptoms of ALS or frontotemporal dementia) showed increased NfL concentrations occurring at least a year before clinical disease.<sup>169</sup> Once disease begins, serum NfL concentrations are stable longitudinally allowing for its use as a pharmacodynamic marker. More recently, incorporating two related plasma micro-RNAs (mir-181a-5p and mir181b-5p) to NfL concentrations improves the survival prognostication (higher concentrations correlated with shorter survival) especially in the patient group with intermediate (59-109 pg/ml) NfL concentrations.<sup>172</sup> Similar approaches of combining protein(s) and/or RNA(s) biomarkers will be useful owing to enhanced prognostic power.

As an easily accessible biofluid, the urine is an attractive option for screening for biomarkers. The p75 neurotrophin receptor is found on the surface of apoptotic motor neurons and Schwann cells. During normal processing, the ecto domain of the p75 molecule (p75<sup>ECD</sup>) is cleaved and becomes detectable in urine. It is elevated in ALS and increases further with disease progression, thereby making it a putative biomarker.<sup>173</sup> The p75<sup>ECD</sup>, unlike neurofilaments, increases at the time of pheno-conversion and not before, thereby serving as a potential marker of pheno-conversion. As a marker of neuroinflammation, chitinases and related proteins (CHIT-1, CHI3L1, CHI3L2) increase in the cerebrospinal fluid as ALS progresses.<sup>174 175</sup> Although chitinases may be a proxy for neuroinflammation, their use as a biomarker in therapies may be hampered by accessibility (poor serum and cerebrospinal fluid correlation) and polymorphisms that may decrease the protein concentration.<sup>176</sup>

## Advances in ALS therapy

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### Multidisciplinary care

The cornerstone of ALS care is an integrative approach because of the clinical and psychosocial complexities.<sup>177 178</sup> A common care model in the US consists of an ALS specialist, nurse, pulmonologist, speech and language pathologist, nutritionist, physical therapist, occupational therapist, and social worker in one clinic visit (as “one stop shop”). Other clinicians with critical roles include a psychiatrist, neuropsychologist, genetics counselor, and gastroenterologist. The ALS team collaborates and seamlessly coordinates care with the primary care clinician and other community or home based health service providers. Additional support is achieved by referral to ALS/MND organizations. This patient centric model of care enhances engagement of patients and care givers in treatment and confers benefits such as improved quality and efficiency of care, access to health and governmental agency services, quality of life, and survival.

### Disease modifying therapies

In the past 20 years, most trials evaluating ALS therapeutics aiming to slow or arrest the neurodegenerative process have failed to show efficacy. These therapies have primarily targeted excitotoxicity, oxidative stress, mitochondrial dysfunction, protein homeostasis, nucleocytoplasmic transport, neuroinflammation, cell death, cytoskeletal integrity, axonal transport, DNA repair, RNA metabolism, and stress granule regulation.<sup>179</sup> As new trials are planned, a collaborative effort has

been made to identify contributors to the failure of studies such as clinical and biological heterogeneity. Critical future steps for the global ALS community to accelerate successful development of ALS therapy include ensuring equity of access, optimizing study design and analysis, endpoint harmonization, and data sharing.

Three disease modifying drugs are approved by the US Food and Drug Administration (FDA) with a primary indication for the treatment of ALS ([table 2](#)). Riluzole, an anti-glutaminergic drug, increases survival and slows the decline in muscle testing score.<sup>[180](#) [181](#)</sup> The most common side effects are asthenia, gastrointestinal symptoms, and an increase in liver enzymes. Edaravone, a free radical scavenger that acts to decrease oxidative stress, modestly slows ALS disease progression.<sup>[182](#) [186](#)</sup> Edaravone is not approved for ALS treatment in Europe, and its role in ALS therapy continues to be a contested topic.<sup>[187](#) [188](#)</sup> The combination of sodium phenylbutyrate and taurursodiol targeting mitochondrial dysfunction, endoplasmic reticulum stress, and cell death, was approved by the FDA in 2022.<sup>[184](#)</sup>

### Pulmonary intervention

Pulmonary system complications are common in ALS, and respiratory failure is the most frequent cause of death.<sup>[189](#)</sup> Pulmonary studies relevant in ALS care include spirometry, nocturnal pulse oximetry, arterial blood gas, polysomnography, maximal inspiratory pressure/maximal expiratory pressure, transdiaphragmatic pressure, and sniff nasal pressure.<sup>[178](#) [189](#) [190](#)</sup> Serial evaluations are essential to identify respiratory muscle weakness and allow for early interventions using non-invasive ventilation, which has been shown to prolong survival with improved quality of life.<sup>[191](#)</sup> Mechanical insufflation-exsufflation is routinely used by ALS patients to augment weak cough to clear airway secretions; however, no systematic study has evaluated the benefits of this intervention. Respiratory muscle training to improve cough and swallowing is an area of active research.<sup>[192](#)</sup>

### Diet and nutritional intervention

Weight loss (specifically fat loss) has been shown to correlate with decline in ALSFRS-R scores, and most patients are advised to adapt their diet to maintain a weight close to their premorbid state.<sup>[193](#)</sup> Weight loss is multifactorial and associated with decreased food intake due to dysphagia, impaired limb dexterity in handling utensils, hypermetabolism (in about 50% of patients),<sup>[194](#)</sup> loss of appetite, and fatigue. Extremes in body mass index (<18, >40) were associated with shorter survival, and best survival was observed for body mass index maintained in the 30-35 range.<sup>[195](#)</sup> The consensus is that the diet should include fiber, carotenes, fruits, and antioxidants.<sup>[196](#)</sup> However, little consensus exists on the high calorie nutritional source—that is, carbohydrates versus polyunsaturated fats. Clinical guidelines recommend discussion of gastrostomy tube insertion for patients who have symptomatic dysphagia, prolonged eating time, negative caloric balance, unintentional weight loss of greater than 5-10%, and, in some cases, declining respiratory status (forced vital capacity approaching 50%).<sup>[189](#)</sup> The benefits of a gastrostomy tube vary depending on proper patient selection, timing of the procedure, careful management of the insertion process, and post-procedure tube management.

ALS patients often ask about over-the-counter supplements and vitamins alone or in combinations. The ALS Untangled ([www.alsuntangled.com](http://www.alsuntangled.com)) initiative has reviewed the evidence for many vitamins and supplements and is an excellent guide for patients, care givers, and clinicians. Unfortunately, most clinical trials have not shown slower ALS progression.<sup>197 198</sup> A recent phase 3 trial of ultrahigh dose methylcobalamin (50 mg) compared with placebo showed a modest slowing in clinical deterioration in treated patients, and evidence suggests that vitamin E may be protective against development of ALS.<sup>199 200</sup>

## Emerging treatments

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The modest effects of the current FDA approved therapeutics for such a devastating disease have spurred a growing pipeline of investigational agents. The development of the ALS platform trial allows for simultaneous testing of multiple agents, using a shared master protocol and central infrastructure.<sup>201</sup> Investigation of at least 50 small molecules with various mechanisms is under way. The successes of therapeutics targeting pathogenic gene expression such as antisense oligonucleotides have led to growing interest in this technology in genetic forms of ALS. This is realized in the accelerated approval of tofersen by the FDA for treatment of SOD1-ALS in April 2023. Several phase 1-2 trials are under way examining the benefits of different antisense oligonucleotides targeting *C9ORF72* and *FUS*. Vectors for gene therapy using adeno-associated virus to reduce SOD1 concentrations are also being explored in a phase 1 trial. Monoclonal antibodies targeting misfolded proteins are being evaluated in phase 2 clinical trials.<sup>202</sup>

## Guidelines

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The American Academy of Neurology (AAN) practice parameters and European Federation of Neurological Societies (EFNS) guidelines review clinical management of ALS.<sup>177 178 189</sup> The AAN parameters provide a comprehensive, systematic, evidence based review of class I-III studies. However, owing to insufficient evidence for the management of certain symptoms (for example, cramps, spasticity, cognitive/behavioral impairment, pain, and dyspnea), no formal recommendations were made in these domains. The EFNS guidelines include book chapters and review papers, and final recommendations were reached by consensus. Both the AAN and EFNS recommend access to a multidisciplinary center and treatment with riluzole. The EFNS guidelines also cover effective communication of the diagnosis and guidelines for genetic testing. Both the AAN and EFNS recommend percutaneous endoscopic gastrostomy placement for symptom progression and weight stabilization before the vital capacity falls below 50% predicted to minimize procedural related risk. Both guidelines recognize non-invasive ventilation to alleviate symptoms of respiratory insufficiency and to prolong survival. The use of invasive mechanical ventilation is discussed, recognizing that this decision varies according to many factors including economic and cultural differences and that, although this prolongs survival, it may not improve quality of life. The AAN practice parameter recognizes the lack of adequate data on drug treatment for cognitive or behavioral impairment in ALS, so no formal recommendations were made.



## Conclusions

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In the past 20 years, considerable progress has been made in basic research on ALS. However, this accumulation of knowledge has been slow to translate into effective therapies, a major source of frustration to patients and care givers. With the inclusion of biomarkers, careful and innovative clinical trial design, and targeting of early disease in pre-symptomatic gene mutation carriers, the field is closer to converting basic science discoveries into disease modifying therapies. For the larger group of patients with sporadic ALS, the hope is that by uncovering gene variants that confer risk, and finding methods to define the predominant mechanism (for example, inflammation versus retro-transposon activation versus oxidative stress pathway) as the driver of disease, a personalized approach similar to that for cancer therapeutics can turn ALS into a chronic disease with limited disability and a dignified life.<sup>203</sup>

### Glossary of abbreviations

AAN—American Academy of Neurology  
ALS—amyotrophic lateral sclerosis  
ALSFRS-R—revised ALS functional rating scale  
ALS-FTD—ALS with frontotemporal dementia  
BOLD—blood oxygen level dependent  
CMAP—compound muscle action potential  
DTI—diffusion tensor imaging  
EFNS—European Federation of Neurological Societies  
EIM—electrical impedance electromyography  
FDA—Food and Drug Administration  
FUS—fused in sarcoma  
HRE—hexanucleotide repeat expansion  
LMN—lower motor neuron  
MND—motor neuron disease  
MRI—magnetic resonance imaging  
MRS—magnetic resonance spectroscopy  
MUNE—motor unit number estimation  
MUNIX—motor unit number index  
NfL—neurofilament light chain  
PCR—polymerase chain reaction  
PET—positron emission tomography  
PLS—primary lateral sclerosis  
PMA—progressive muscular atrophy  
SOD1—superoxidase dismutase 1  
TDP-43—transactive response DNA binding protein 43  
TMS—transcranial magnetic stimulation  
UMN—upper motor neuron

## Questions for future research

How can diagnostic criteria for amyotrophic lateral sclerosis (ALS) be improved to fully capture motor and cognitive dysfunction?

What are the genetic contributors to sporadic ALS?

Can diminishing inflammation in the brain and spinal cord of patients with ALS result in slowing of disease progression?

What are the major factors driving disease progression, and can they be modified?

What determines disease presentation (ALS versus frontotemporal dementia (FTD) versus ALS-FTD)?

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## Notes

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Series explanation: State of the Art Reviews are commissioned on the basis of their relevance to academics and specialists in the US and internationally. For this reason they are written predominantly by US authors

## Notes

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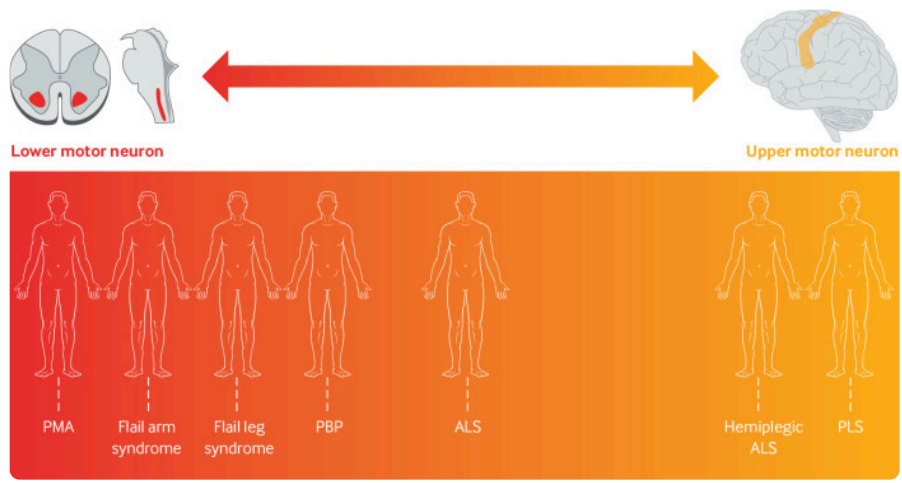
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## Figures and Tables

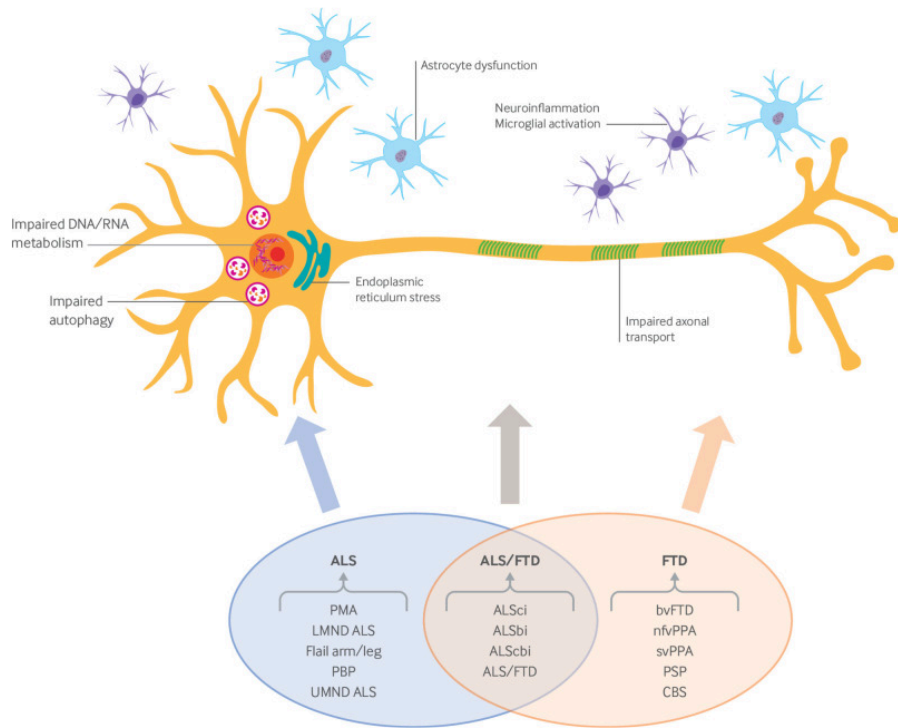
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Fig 1



Amyotrophic lateral sclerosis (ALS) phenotypic spectrum. PBP=progressive bulbar palsy; PLS=primary lateral sclerosis; PMA=progressive muscular atrophy. Created using BioRender.com

Fig 2



Amyotrophic lateral sclerosis with frontotemporal dementia (ALS-FTD) clinical syndromes and disease mechanisms contributing to neurodegeneration. ALS=amyotrophic lateral sclerosis; ALSbi=ALS with behavioral impairment; ALScbi=ALS with combined cognitive and behavioral impairment; ALSci=ALS with cognitive impairment; bvFTD=behavioral variant FTD; CBS=corticobasal syndrome; FTD=frontotemporal dementia; LMND=lower motor neuron predominant; nvPPA=non-fluent variant primary progressive aphasia; PBP=progressive bulbar palsy; PLS=primary lateral sclerosis; PMA=progressive muscular atrophy; PSP=progressive supranuclear palsy; svPPA=semantic variant primary progressive aphasia; UMND=upper motor neuron predominant

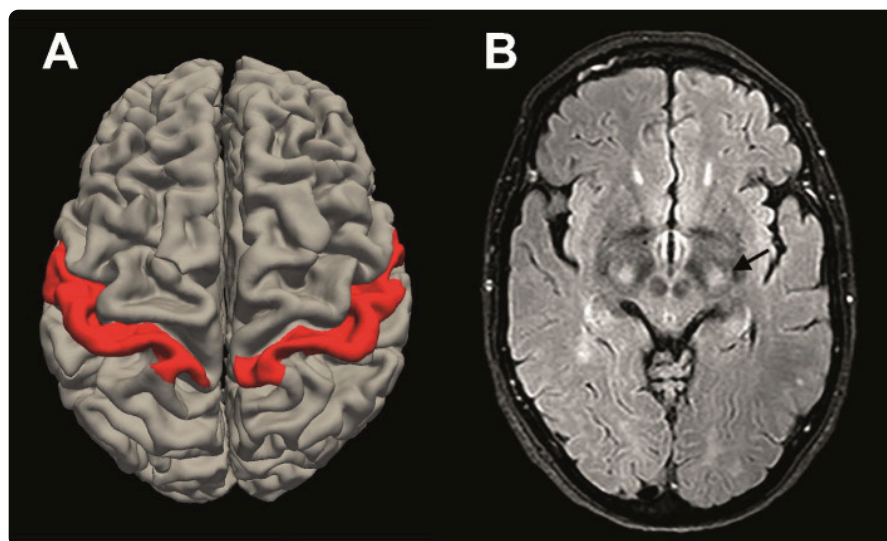
**Table 1**

Rare causative genes for amyotrophic lateral sclerosis (ALS)

<b>Gene (OMIM number)</b>	<b>Protein</b>	<b>Associated clinical diagnosis/feature</b>
<b>RNA/DNA binding proteins (involved in pre-mRNA processing, metabolism, and transport)</b>		
<i>TARDBP</i> (*605078)	TAR DNA binding protein 43 (TDP-43)	ALS with or without FTD; FTD
<i>FUS</i> (*137070)	Fused in sarcoma protein	ALS with or without FTD; hereditary essential tremor
<i>hnRNPA1</i> (*164017) and <i>hnRNPA2B1</i> (*600124)	Heterogeneous nuclear ribonuclear protein A1 and A2B1	ALS; inclusion body myopathy with early onset Paget's disease with or without frontotemporal dementia
<i>MATR3</i> (*164015)	Matrin 3	ALS with or without cognitive impairment or dementia; distal myopathy
<i>ANG</i> (*105850)	Angiogenin	Frequent bulbar onset ALS; co-existing parkinsonism with FTD
<b>Genes that encode for structural proteins (cytoskeleton proteins)</b>		
<i>TUBA4A</i> (*191110)	Tubulin- $\alpha$ 4A ( $\alpha$ tubulin)	ALS with or without FTD
<i>ANXA11</i> (*602572)	Annexin A11	Later onset ALS (average 67 years); inclusion body myopathy and brain white matter abnormality
<i>PRPH</i> (*170710)	Peripherin	ALS
<i>DCTN1</i> (*601143)	Dynactin 1	Distal motor neuronopathy with vocal paresis
<i>PFN1</i> (*176610)	Profilin 1	ALS
<i>KIF5a</i> (*602821)	KIF5a (kinesin family member 5A)	Also implicated in HSP-10, CMT
<b>Loss-of-function mutations in genes encoding proteins important for protein degradation or autophagy pathways</b>		
<i>UBQLN2</i> (*300264)	Ubiquilin-2	ALS with or without FTD; X linked dominant familial ALS; onset younger in males than females
<i>SQSTM1</i> (*601530)	p62/sequestosome 1	FTD with or without Paget's disease of the bone; distal myopathy with rimmed vacuoles
<i>OPTN</i> (*602432)	Optineurin	ALS with or without FTD; allelic with primary open angle glaucoma
<i>VCP</i> (*601123)	Vasolin containing protein	FTD with or without ALS; inclusion body myopathy with Paget's disease of bone and

CMT=Charcot-Marie-Tooth disease; FTD=frontotemporal dementia; HSP=hereditary spastic paraplegia.

Fig 3



Neuroimaging changes in amyotrophic lateral sclerosis. A: motor cortex (red) atrophy. B: axial T2-FLAIR (fluid attenuated inversion recovery sequence) magnetic resonance image at level of midbrain, showing hyperintensity in cerebral peduncles corresponding to corticospinal tracts (black arrow)

**Table 2**

Clinical trials leading to US Food and Drug Administration approved therapies for amyotrophic lateral sclerosis (ALS)

Drug, study	Design	No of participants	Primary outcome(s)	Result
Riluzole <sup>180</sup>	Prospective, double blind, randomized, placebo controlled	155	1: survival; 2: rate of change in functional status (limb function, bulbar function, muscle testing score)	1: 45/78 (58%) in placebo group remained alive v 57/77 (74%) in riluzole group at 12 months (P=0.014); 2: Rate of deterioration for limb function, bulbar function, and muscle strength was slower in riluzole group, but only statistically significant for muscle testing score (P=0.028)
Riluzole <sup>181</sup>	Double blind, randomized, placebo controlled	959	Survival without tracheostomy on 100 mg dose	122 (50.4%) in placebo group and 134 (56.8%) in riluzole group survived; adjusted risk 0.65 (P=0.002)
Edaravone <sup>182</sup>	Randomized, double blind, placebo controlled	137	Change in ALSFRS-R score from baseline to 24 weeks	Edaravone -5.01 v placebo -7.50; least squares mean difference 2.49 (95% CI 0.99 to 3.98; P=0.001)
Edaravone <sup>183</sup>	Post hoc analysis of 24 week randomized, placebo controlled study followed by 24 weeks of open label extension study	88	Change in ALSFRS-R score at week 48 in patients with FVC ≥80% v <80% (FVC assessed at week 24)	FVC >80% subgroup: -7.63 v -9.69; difference 2.05 (95% CI 0.16 to 3.94; P=0.034). FVC <80% subgroup: -10.26 v -15.20; difference 4.94 (95% CI 1.64 to 8.25; P=0.004).
Sodium phenylbutyrate- controlled trial (CPB)	Randomized, double blind; 2:1 (drug:placebo)	137	Mean rate of decline in ALSFRS-R at 24 weeks	PB-TUDCA v placebo: -1.24/month v -1.66/month; difference 0.42

ALSFRS-R=revised ALS functional rating scale; CI=confidence interval; FVC=forced vital capacity.

\*Did not meet primary endpoint.