Review

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Advances in treating amyotrophic lateral sclerosis: insights from pathophysiological studies

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Amyotrophic lateral sclerosis (ALS) is the most frequently occurring of the neuromuscular degenerative disorders, with a median survival time of 3–5 years. The pathophysiological mechanisms underlying ALS are multifactorial, with a complex interaction between genetic factors and molecular pathways. To date 16 genes and loci have been associated with ALS, with mutations in DNA/RNA-regulating genes including the recently described *c9orf72* (chromosome 9 open reading frame 72) gene, suggesting an important role for dysregulation of RNA metabolism in ALS pathogenesis. Further, dysfunction of molecular pathways, including glutamate-mediated excitotoxicity, has been identified in sporadic and familial ALS, indicating the existence of a common pathogenic pathway. These pathophysiological insights have suggested novel therapeutic approaches, including stem cell and genetics-based strategies, providing hope for feasible treatment of ALS.

Advances in ALS

ALS, colloquially known as Lou Gehrig's disease, is a rapidly progressive and universally fatal neurodegenerative disorder of the human motor system, first described in the mid-19th century [1]. Although ALS is heterogeneous in age and site of disease onset, as well as rate of disease progression, clinically ALS is characterized by progressive neurological deterioration and coexistence of upper and lower motor neuron signs, suggesting that ALS is 'one disease'. Despite the clinical heterogeneity, median survival time of ALS patients is 3–5 years [2].

Understanding disease pathogenesis appears to be central for future development of diagnostic and therapeutic strategies in ALS. Over the past decade evidence has emerged of unique pathophysiological processes, such as glutamate-mediated excitotoxicity, resulting in the development of novel diagnostic investigations and potential therapeutic strategies. Advances in genetics, including the recently discovered *c9orf72* gene, have radically changed the pathological mind-set from ALS being classified as a neuromuscular disease to one forming a continuum with frontotemporal dementia [3,4]. In the absence of curative therapies, recent advances in ALS pathophysiology provide hope for the development of novel neuroprotective strategies. Establishment of multidisciplinary therapeutic approaches and the development of population-based registries are beginning to yield vital insights into ALS phenotypes and the unpredictable rate of inter-subject disease progression, as well as the development of therapeutic guidelines for improved symptomatic management of ALS patients. This review aims to discuss current advances in the understanding of the pathogenesis and management of ALS.

Clinical features and diagnosis

ALS exhibits a diverse and complex clinical phenotype that is crucial to understanding disease pathophysiology and diagnosis. Clinically, ALS is characterized by the coexistence of upper (UMN) and lower motor neuron (LMN) signs encompassing multiple body regions, with evidence of progressive deterioration [5]. Atypical ALS phenotypes include the 'pure' LMN-type progressive muscle atrophy (PMA), 'pure' UMN-type primary lateral sclerosis (PLS), and predominant bulbar palsy (PBP). One-third of PMA cases develop UMN dysfunction [6,7] whereas PLS patients may develop LMN signs within 4 years of disease onset [8]. The PBP phenotype remains localized within the bulbar region for a prolonged period (>6 months) and is characterized by greater female predominance and UMN bulbar dysfunction, although clinical features of ALS typically develop in the upper and lower limbs [9]. Although survival is typically prolonged in these usual phenotypes, the mortality rates for the generalized forms of PMA appear similar to ALS [6]. Given the varied clinical phenotypes, detailed investigations are essential before establishing a diagnosis of ALS, including neurophysiological (Figure 1), laboratory investigations, and infrequently nerve and muscle biopsies combined with neuroimaging approaches to exclude mimic disorders (Table 1).

The clinical hallmark of ALS remains the identification of UMN and LMN signs in multiple body regions. Lower

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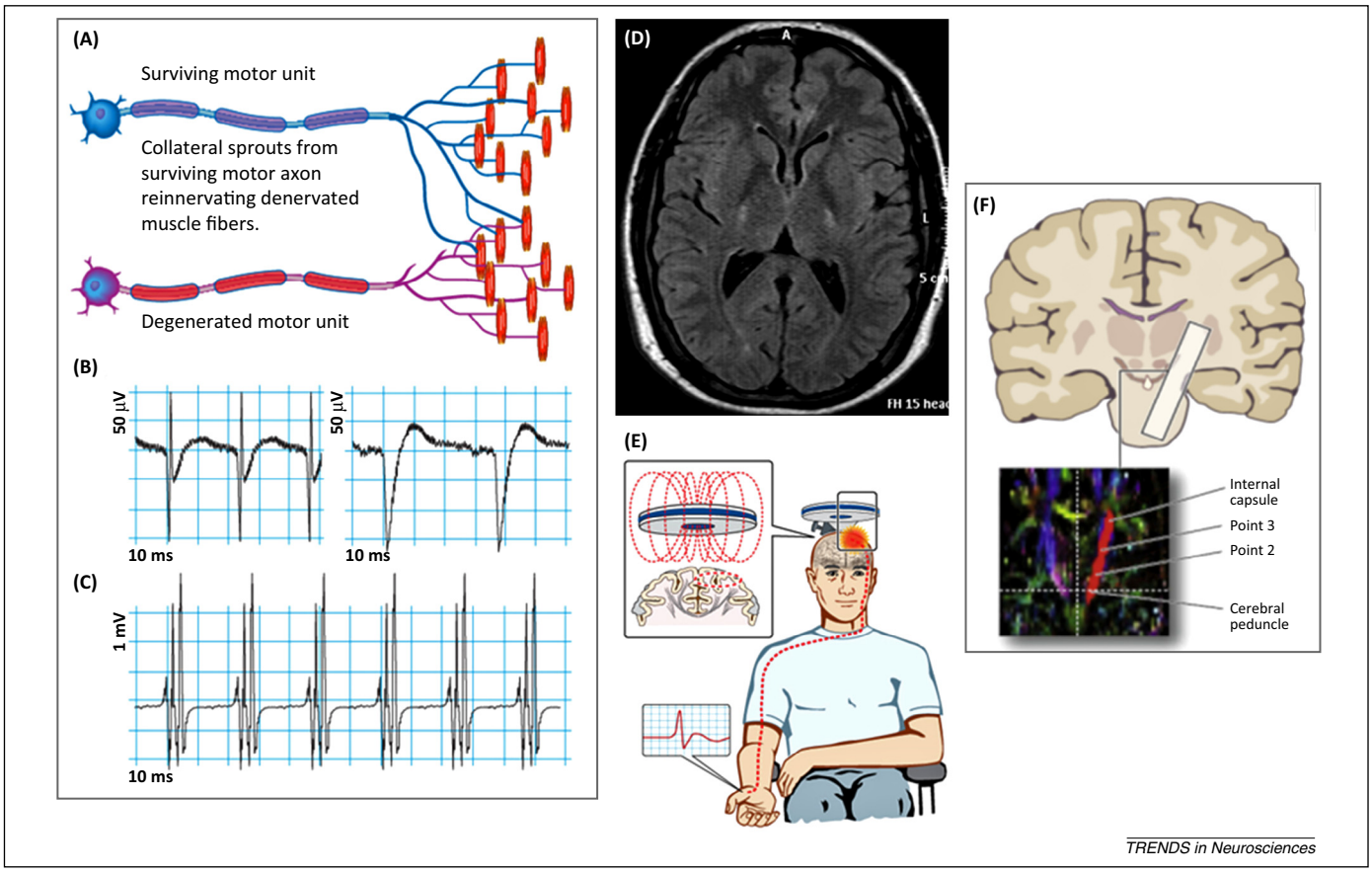

Figure 1. Diagnostic techniques in amyotrophic lateral sclerosis (ALS). **(A)** Ongoing degeneration of motor neurons accompanied by collateral sprouting of surviving motor neurons results in classical electromyography findings including **(B)** ongoing denervation (fibrillation potentials and positive sharp waves) accompanied by **(C)** chronic neurogenic changes (large amplitude, long-duration, polyphasic motor unit action potentials with reduced voluntary recruitment). Although the electromyogram (EMG) changes may not be specific for ALS when found in isolation, the sensitivity and specificity of EMG findings as they conform to the Awaji–Shima criteria has been recently established [100]. In addition, techniques for assessing upper motor neuron function including **(D)** conventional magnetic resonance imaging (MRI), **(E)** transcranial magnetic stimulation (adapted, with permission, from [55]), and **(F)** diffusion tensor imaging may be important diagnostic aids in ALS (adapted, with permission, from [101]).

motor neuron signs are clinically characterized by fasciculations, with muscle wasting and weakness, whereas UMN signs may be heralded by slowness of movement, increased tone, and hyper-reflexia; extensor plantar responses are evident in 47% of patients [10]. The majority of ALS patients present with limb-onset disease (65–75%) [11], with preferential wasting and weakness of the thenar muscles, termed the split-hand [12]. Although fasciculations are a cardinal feature of ALS, they are infrequently the presenting symptom [13]. Patients presenting solely with fasciculations and muscle cramping should be monitored because these may infrequently progress to develop ALS [14].

Bulbar-onset disease, evident in 20% of cases, is characterized by flaccid or spastic dysarthria, dysphagia, hoarseness, tongue wasting, weakness, and fasciculations, as well as emotional lability and pathologically brisk jaw reflexes [5]. Dysphagia may potentially result in aspiration pneumonia, malnutrition, and weight loss, all adverse prognostic features [15]. Respiratory dysfunction develops in advanced stages of ALS, ultimately resulting in terminal respiratory failure [16], although this is rarely the presenting symptom [17].

In addition to 'pure' motor symptoms, subtle cognitive abnormalities may be evident in up to 50% of ALS patients [18,19], characterized by executive dysfunction, language and memory impairment, together with behavioral abnormalities, and these may precede the onset of motor symptoms [18–20]. Recognition of cognitive dysfunction has implication for vital management of ALS because these symptoms may adversely impact on patient compliance and decision-making abilities. At the extreme end of the spectrum, frontotemporal dementia (FTD) may develop in up to 15% of ALS patients [18,21], and is clinically characterized by executive and language dysfunction, irrational behavior, personality changes, apathy, poor insight, loss of empathy, irritability, and disinhibition [22]. The presence of psychiatric features in the setting of FTD-ALS may be the harbinger of the *c9orf72* expansion [22].

ALS pathophysiology

Although the mechanisms underlying ALS pathogenesis remain to be fully elucidated, emerging evidence suggests the importance of genetic factors and dysfunction of vital molecular pathways (Figure 2). A genetic etiology has been identified in up to 20% of apparently sporadic and 60% of familial ALS cases, with at least 16 genes and genetic loci being implicated in ALS pathogenesis [23]. Importantly, these genetic breakthroughs have shed light on the site of

Table 1. Differential diagnosis for ALS[a,b]

Differential diagnosis	Investigations
Disorders of motor neurons and nerves	
Spinal muscular atrophy	Survival motor neuron (SMN) gene
X-linked spinobulbar muscular atrophy (Kennedy's disease)	CAG triplet repeat-androgen receptor on the X chromosome
Poliomyelitis or post-polio syndrome	EMG/clinical history
Tay–Sachs disease	Hexosaminidase A deficiency
Multifocal motor neuropathy	NCS, anti-GM1 antibodies
CIDP	NCS, SSEP
Cramp-fasciculation syndrome	NCS, voltage-gated K^+ channel antibodies
Hereditary spastic paraparesis	Genetic testing, clinical history, EMG
Hereditary motor neuropathy	NCS, EMG, clinical history
Paraneoplastic syndrome	Paraneoplastic serology
Heavy metal poisoning	Heavy metal screen
Mononeuritis multiplex	NCS, EMG, nerve biopsy
Disorders of the neuromuscular junction	
Myasthenia gravis	RNS, SFEMG, serology, search for thymoma
Lambert–Eaton myasthenic syndrome	RNS, SFEMG, serology, search for primary tumor
Disorders of muscle	
Inclusion body myositis[c]	Muscle biopsy (inclusion bodies)
Polymyositis and dermatomyositis	Muscle biopsy
Muscular dystrophies	Muscle biopsy, immunohistochemistry, genetic testing
	Thyroid function tests
Thyrotoxicosis	Parathyroid hormone, Ca^{2+}
Hyperparathyroidism	Muscle biopsy
Polyglucosan body disease	EMG, genetic testing
Myotonia and neuromyotonia	
Structural lesions of brain and spine	
Cervical disk disease[c]	EMG, MRI spine
Radiculoplexopathy	EMG, MRI spine
Syringomyelia/syringobulbia	MRI spine
Monomelic amyotrophy (Hirayama's disease)	MRI cervical spine-flexion and extension
Spinal cord tumors	
Multiple sclerosis	MRI spine
	MRI brain/spinal cord, SEEP, CSF oligoclonal bands
HIV/HTLV1,2 myelopathy	Serology
Lyme disease	Serology[d]
Subacute combined degeneration	Vitamin B12
Tabes dorsalis	Syphilis serology

[a]A multitude of disorders could potentially mimic ALS. Nerve conduction studies (NCS), electromyography (EMG) and somatosensory evoked potentials (SSEP) should be initially utilized to differentiate ALS from mimic disorders. Low-frequency (3 Hz) repetitive nerve stimulation (RNS) and single-fiber EMG (SFEMG) together with serology, including acetylcholine receptor antibodies, anti-muscle specific tyrosine kinase (MUSK) antibodies, and Lrp4 antibodies, should be utilized to diagnose myasthenia gravis. For Eaton–Lambert syndrome, high-frequency RNS (20 and 50 Hz), and serology for the P/Q voltage-gated Ca^{2+} channels should be performed.

[b]Additional abbreviations: CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; GM1, monosialotetrahexosylganglioside; HTLV, human T cell lymphotropic virus; MRI, magnetic resonance imaging; SEEP; signal enhancement by extravascular water protons.

[c]Inclusion body myositis and cervical disk disease are the two most frequent confounding diagnoses in ALS. In addition, heavy metal poisoning is regarded as a controversial cause of ALS, whereas screening for paraneoplastic serology, anti-GM1 antibodies, and hexosaminidase-A deficiency is not routinely performed by most ALS physicians.

[d]Testing for Lyme disease may include two-tiered algorithm with a whole cell sonicate (WCS) enzyme immunoassay (EIA), followed by IgM/IgG Western immunoblots or a two-tiered strategy using WCS EIA as the first step, followed by EIA using the C6 peptide of the *Borrelia burgdorferi* variable-major protein-like sequence lipoprotein.

disease onset, a controversial aspect of ALS pathogenesis with clear diagnostic and therapeutic implications (Box 1).

The C9orf72 hexanucleotide expansion

A major advance in the understanding of ALS pathogenesis occurred with the discovery of the dominantly inherited *c9orf72* gene [increased hexanucleotide repeat expansion (GGGGCC)], which appears to underlie over 40% of familial and 20% of sporadic ALS cases [3,4], although subsequent studies have established a frequency of 4.1–8.3% of *c9orf72* mutations in apparently 'sporadic' ALS cases [24]. This monumental discovery has radically altered the understanding of ALS pathogenesis, implying that ALS is a multisystem neurodegenerative disorder rather than a pure neuromuscular disease. Underscoring this notion are findings that the *c9orf72* hexanucleotide expansions are causative in ALS and frontotemporal dementia [3,4]. The accumulation of TDP-43 (TAR DNA-binding protein 43, also known as TARDBP), together with p62-positive TDP-43-negative inclusions, in hippocampal and cerebellar neurons appears to be a neuropathological hallmark of *c9orf72*-associated ALS and FTD [25], suggesting the existence of a common pathophysiological pathway.

The mechanisms by which the *c9orf72* gene expansion leads to neurodegeneration in ALS remains to be elucidated fully [3,4], although three potential pathogenic mechanisms have been proposed, including (i) haploinsufficiency, (ii) repeat RNA-mediated toxicity, and (iii) dipeptide protein toxicity related to repeat-associated non-ATG (RAN) translation of the expanded *c9orf72* gene [26]. Evidence for haploinsufficiency is suggested by studies reporting a reduction in the *c9orf72* short and long isoforms in ALS

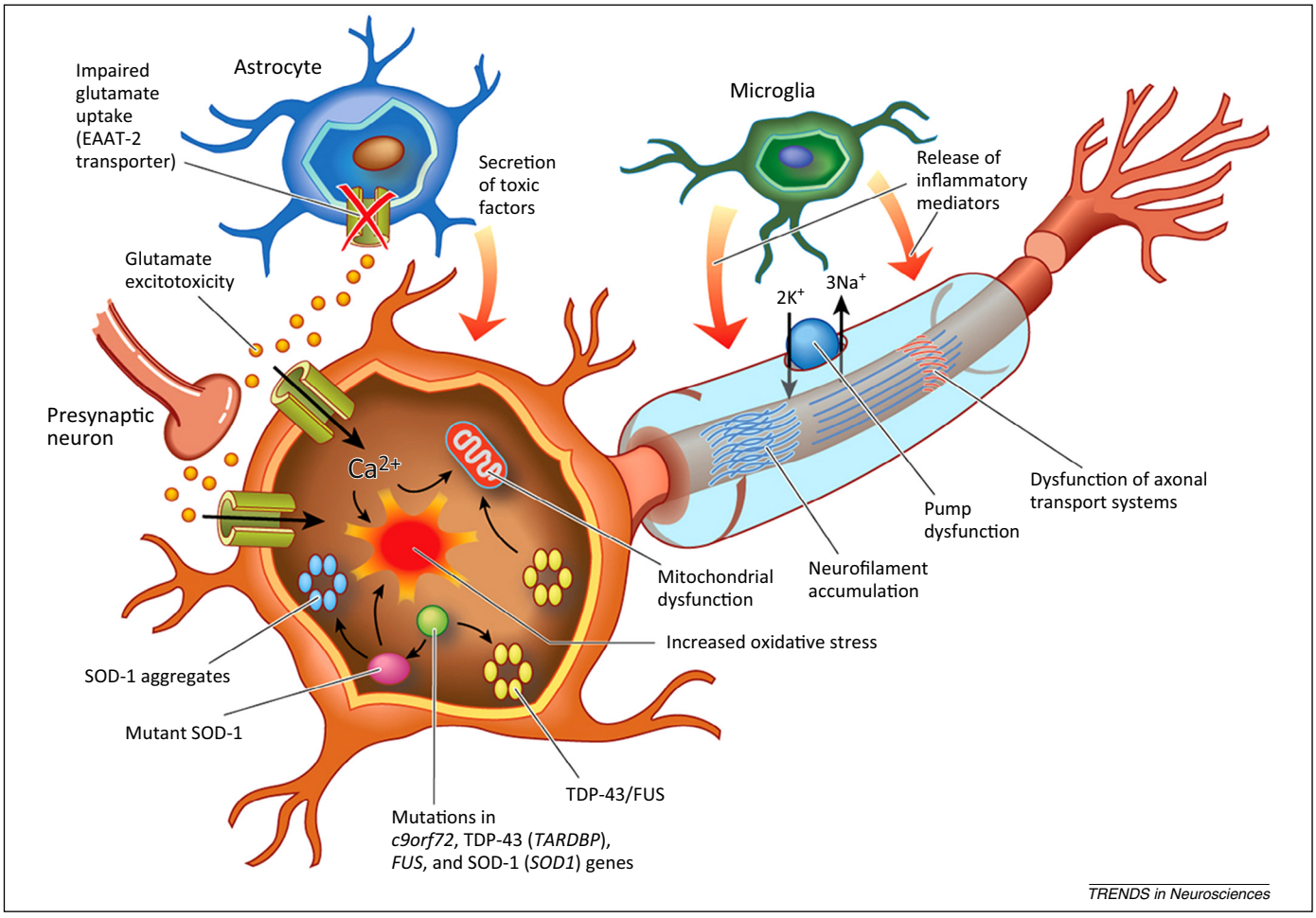

Figure 2. Pathophysiology of amyotrophic lateral sclerosis (ALS). The pathophysiological mechanisms underlying neurodegeneration in ALS appear to be multifactorial with evidence of a complex interplay between molecular and genetic pathways. Dysfunction of the astrocytic excitatory amino acid transporter 2 (EAAT2) results in reduced uptake of glutamate from the synaptic cleft and thereby glutamate excitotoxicity. Glutamate-induced excitotoxicity results in increased influx of Na^+ and Ca^{2+} ions and ultimately neurodegeneration through activation of Ca^{2+}-dependent enzymatic pathways. In addition, glutamate excitotoxicity results in the generation of free radicals which in turn contribute to neurodegeneration. Mutations in *c9orf72*, TDP-43 (*TARDBP*) and *FUS* result in dysregulated RNA metabolism that ultimately leads to the formation of intracellular aggregates which are harmful to neurons. Of further relevance, mutant SOD-1 enzymes increase oxidative stress, induce mitochondrial dysfunction, form intracellular aggregates, and adversely affect neurofilament and axonal transport processes. Activation of microglia results in secretion of proinflammatory cytokines, producing further toxicity. Abbreviations: c9orf72, chromosome 9 open reading frame 72; FUS, fused in sarcoma; SOD-1, superoxide dismutase 1 (SOD1); TDP-43, TAR DNA-binding protein 43 (TARDBP).

patients [3,4], although a reduction in the corresponding c9orf72 protein has yet to be established. In addition, reduced expression of the *c9orf72* transcript in the zebrafish model of ALS resulted in motor axonal degeneration with locomotion deficit, providing additional support for haploinsufficiency as a factor in ALS pathogenesis [27].

Of further relevance, RNA-mediated toxicity has also been proposed as a potential mechanism. Such a process was inferred from observations of intranuclear RNA foci containing *c9orf72* hexanucleotide repeats [4], and is supported by findings that specific RNA-binding proteins associate with the *c9orf72* expansion, resulting in the formation of intranuclear and cytoplasmic inclusions [28]. In keeping with RNA toxicity, recent studies have demonstrated the formation of r(GGGGCC) RNA G-quadruplex structures that could sequester transcription factors (ASF/SF2 and hnRNPA1) crucial in DNA/RNA metabolism [29]. More recently, studies utilizing induced pluripotent stem cell differentiated neurons from *C9orf72* patients have provided additional support for RNA toxicity and, importantly, established that the pathological changes were mitigated by antisense oligonucleotide therapeutic approaches [30].

In addition, RAN translation of the *c9orf72* expansion has also been proposed as a potential pathogenic mechanism [31]. Specifically, RAN translation results in generation of insoluble dipeptides (anti-C9RANT) which form intraneuronal (nuclear and cytoplasmic) inclusions and appear to be specific for *c9orf72*-associated ALS/FTD [31]. Given that neuronal degeneration and dysfunction may result from the accumulation of insoluble proteins, and that C9RANT-positive pathology appears to be specific for *c9orf72*-related ALS/FTD, novel therapeutic strategies aimed at modulating such a process may prove useful.

Transactive-region DNA-binding protein gene (TARDBP) and fused in sarcoma (FUS)

Mutations in *TARDBP* [32] and *FUS* [33], that encode DNA/RNA-processing polypeptides, have also been linked with development of ALS, representing 4–6% of familial and 0.7–2% of sporadic ALS [23]. TDP-43/TARDBP and FUS are ubiquitously expressed proteins involved in DNA

Box 1. Controversial aspect of ALS pathogenesis: Site of disease onset?

Although the primacy of upper motor neuron dysfunction in ALS pathogenesis was first proposed by Charcot [1], the issue of where ALS begins remains a matter of debate. Resolution of this very important issue is of pathophysiological, diagnostic, and therapeutic significance in ALS. A 'dying-forward' hypothesis was first proposed by Eisen and colleagues in 1992 [72] wherein it was proposed that ALS is primarily a disorder of corticomotoneurons, which mediate anterior horn cell degeneration via an anterograde glutamate excitotoxic mechanism. Support for such a dying-forward hypothesis was inferred from clinical observations that motor neurons lacking a monosynaptic connection with corticomotoneurons, such as the oculomotor, abducens, and Onuf's nuclei, are typically spared in ALS, the absence of pure lower motor neuron forms of ALS, and the absence of naturally occurring animal models of ALS, ascribed to a paucity of corticomotoneuronal–anterior horn cell connections. In addition, TMS studies documenting that cortical hyperexcitability precedes the clinical onset of familial ALS [70], and the effectiveness of the antiglutaminergic agent riluzole [74,81], provide additional support. In keeping with a cortical origin of ALS is the now-accepted view that ALS and frontotemporal dementia (FTD) represent an overlapping continuum of the same disorder [102], an observation underscored by recent genetic findings establishing that increased hexanucleotide repeat expansion in the first intron of the *C9orf72* gene on chromosome 9p21 is associated with both ALS and FTD [3,4]

By contrast, a 'dying-back' hypothesis proposed that ALS begins within muscle cells or the neuromuscular junction. Specifically, this hypothesis proposes that there is a deficiency of specific motor neurotrophic factors, which are normally released by postsynaptic cells and retrogradely transported up the presynaptic axon to the cell body where they exert neurotrophic effects. Observations that synaptic denervation precedes the onset of anterior horn cell degeneration, and that accumulation of mutant SOD-1 proteins in the Schwann cells may mediate synaptic denervation, provides support for the dying-back hypothesis. However, to date no motor neuron trophic factors have been identified.

Of further relevance, it has also been proposed that upper and lower motor neuron degeneration may occur as independent processes. Neuropathological studies provided support for this 'independent degeneration' hypothesis [103,104]. These morphological techniques, however, may be confounded by the anatomical and functional complexity of the corticomotoneuronal system. In particular, there remains considerable variability in the corticomotoneuronal to anterior horn cell ratio, owing to synaptic changes, and attempts to correlate upper and lower motor neurons on autopsy studies may be problematic.

repair, regulation of RNA transport, translation, splicing, microRNA biogenesis, and the formation of stress granules [26]. To date approximately 50 mutations have been identified in each gene, and most mutations are dominantly inherited [32,34]. Mutations in *TARDBP* are located within the C-terminal glycine-rich domain of the protein [32], whereas *FUS* mutations are located in the C-terminal nuclear localization signal domain which appears to be important for translocation of the FUS protein into the nucleus [34]. The mutant proteins (TDP-43 and FUS) are redistributed from the nucleus to the cytoplasm, resulting in toxicity.

Although the pathophysiological mechanisms by which *TARDBP/FUS* gene mutations result in neurodegeneration remain to be defined fully, emerging evidence suggests multiple mechanisms including gain of toxicity, loss of nuclear function, and the formation of large stress granules [26]. Support for a toxic gain of function has been provided by studies in transgenic mouse models wherein increased expression of the mutated TDP-43 proteins leads to neurodegeneration through dysfunction of cellular organelles and proteins [35]. The severity of cortical and spinal motor neuron degeneration appears to be proportional to TDP-43 protein levels [35], suggesting a potential role for TDP-43 in regulating disease severity. Alternatively, loss of nuclear TDP-43 accompanied by accumulation of TDP-43 aggregates in the cytoplasm has been well established in ALS patients [36], implying a potential role for a TDP-43 loss of nuclear function mechanism in ALS pathogenesis. Emerging evidence from transgenic mouse models provides support for the notion that inactivation of the *TARDBP* gene leads to the development of ALS [37]. As with TDP-43, the finding of cytoplasmic FUS-positive inclusions in ALS patients [33,34] also implies loss of nuclear function as a potential pathogenic mechanism, and is supported by FUS expression studies in transgenic mouse models [38]. Conversely, a toxic-gain of function has also been inferred from FUS expression studies [39].

Of further relevance, TDP-43 and FUS associate with cytoplasmic stress granules [26]. Specifically, stress granules function to suppress mRNA translation temporarily and store pre-RNA complexes during periods of cellular stress, thereby safeguarding the coded RNA information from deleterious chemicals [26]. Pathological TDP-43 and FUS mutant proteins appear to exhibit a greater propensity to associate with cytoplasmic stress granules and form larger stress granules with altered dynamics [33,40]. Although the mechanisms by which altered stress granule dynamics might induce neuronal degeneration in ALS remain to be elucidated fully, sequestration of RNA-binding proteins and repression of RNA translation, together with the formation of pathological inclusions, have been proposed as potential mechanisms [40,41].

Copper/zinc superoxide dismutase-1 (SOD-1) gene

Mutations in the SOD-1 gene (*SOD1*) heralded the genetic age for ALS [42]. To date 166 SOD-1 mutations have been reported, underlying 14–23% of familial and 1–7% of sporadic ALS cases [23]. Intra- and interfamilial variations in penetrance, age, and site of disease onset, rate of disease progression, and survival have been reported for most SOD-1 mutations, with approximately 50% of patients expressing the disease by age 43 and more than 90% by 70 years [43].

The pathophysiological mechanisms by which SOD-1 gene mutations lead to neurodegeneration remain enigmatic [44]. Aberrant biochemical activity of the SOD-1 enzyme (toxic gain of function) has been suggested as a potential pathogenic mechanism [45]. Specifically, SOD-1 mutations may lead to increased production of hydroxyl and free radicals [46], as well as nitration of tyrosine residues on proteins [47]. Evidence for oxidative damage has been inferred from pathological studies in ALS patients [48] and transgenic SOD-1 mouse models [49]. Although oxidative damage seems to be an attractive pathogenic mechanism, findings of normal SOD-1 activity

in patients harboring particular SOD-1 mutations [50], an absence of correlation between dismutase activity and disease severity [51], and lack of beneficial effects of antioxidants in ALS patients [52] all suggest a minor role for oxidative stress in SOD-1-related ALS pathogenesis.

Conformational instability of the SOD-1 peptide, resulting in formation of intracellular aggregates, has also been proposed as a pathogenic mechanism. Importantly, disease severity in patients with SOD-1 mutations appears to correlate with instability of the mutant SOD-1 protein [53]. The mechanisms by which conformation changes in SOD-1 protein lead to neurodegeneration remain to be determined, although co-aggregation of essential cellular components or induction of aberrant catalysis by misfolded SOD-1 mutant proteins have been proposed as potential processes [54].

Glutamate-mediated toxicity

There has been significant progress in uncovering of key molecular pathways that underlie ALS pathogenesis. Glutamate-mediated excitotoxicity appears to be an important pathophysiological process in familial and sporadic forms of ALS [55]. Glutamate exerts effects through an array of ionotropic and metabotropic postsynaptic receptors [56], with excessive activation of ionotropic receptors, including *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors, leading to neurodegeneration through activation of Ca^{2+}-dependent enzymatic pathways [57]. Glutamate-mediated excitotoxicity also results in oxidative stress, leading to neurodegeneration through injury of intracellular organelles and upregulation of proinflammatory mediators [58].

Support for a glutamate-mediated excitotoxic process in ALS has been provided by animal and human studies [59,60]. Reduced expression and activity of the astrocytic glutamate transporter, excitatory amino acid transporter 2 (EAAT-2), were identified in the motor cortex and spinal cord of ALS patients [61] and the transgenic SOD-1 mouse model [62]. Activation of caspase-1, an inhibitor of the EAAT-2 transporter, was reported in the SOD-1 mouse model before onset of motor neuron degeneration and clinical features of ALS [63]. Overexpression of EAAT-2 appeared to be neuroprotective [64], whereas downregulation of EAAT-2 accelerated disease progression [65]. The loss of EAAT-2 appears to delay motor neuron degeneration rather than being a primary event, and other mechanisms, such as SOD-1 aggregation and caspase-3 activation, appear to be important [66].

On the postsynaptic side, increased expression of Ca^{2+}-permeable AMPA receptors containing an unedited GluR2 subunit has been reported in ALS [67]. The GluR2 subunit regulates the Ca^{2+} permeability of AMPA receptors, and absence of this subunit increases the Ca^{2+} permeability of AMPA receptors [68]. This editing defect appears to be specific for ALS, potentially increasing the susceptibility of motor neurons to glutamate excitotoxicity [69]. Importantly, motor neurons in ALS appear to exhibit reduced Ca^{2+}-buffering capacity, thereby rendering these more vulnerable to degeneration [67].

Transcranial magnetic stimulation (TMS) studies have identified cortical hyperexcitability in sporadic and familial ALS, and there is evidence that hyperexcitability precedes the development of clinical features in familial ALS [70,71]. Glutamate-induced excitotoxicity could potentially mediate motor neuron degeneration via an anterograde 'dying forward' process [72], a notion supported by some TMS and transgenic SOD-1 mouse studies [55,73]. The neuroprotective effects of riluzole, a glutamate antagonist, provide additional support for a pathogenic role of glutamate excitotoxity in ALS [74]. These neuroprotective benefits appear to be modest, potentially accounted for by partial normalization of cortical hyperexcitability [75], and may suggest that glutamate toxicity is not the primary cause of neurodegeneration in ALS.

Other molecular mechanisms

Structural and functional abnormalities of mitochondria, impairment of axonal transport systems and endosomal trafficking, together with neuroinflammation and induction of the endoplasmic reticulum stress response, have all been implicated in ALS pathogenesis (Figure 2) [58]. Although these mechanisms contribute to neurodegeneration, they appear to be secondary events in ALS.

Non cell autonomous processes

An emerging concept in ALS pathogenesis pertains to non cell autonomous processes, whereby neighboring glial cells mediate motor neuron cell death [76]. Studies in transgenic mouse models reported that modulation of mutant SOD-1 expressed in microglia slowed disease progression [76]. In addition, astrocytes expressing the mutant SOD-1 exerted toxic effects in cultured primary motor neurons [77], and silencing of mutant SOD-1 genes in astrocytes significantly slowed disease progression [78]. Importantly, non-neuronal cells appear to be crucial in regulating disease progression rather than initiating motor neuron disease [76].

The mechanisms by which non-neuronal cells exert toxicity remain unclear, although multiple interacting mechanisms appear to be responsible. Specifically, impairment of passive properties of astrocytes, such as the uptake or recycling of neurotransmitters and the regulation of extracellular ion homeostasis, accompanied by activation of microglia cells leading to increased secretion of neurotoxic agents, such as glutamate and proinflammatory cytokines, appear to be important mechanisms [79].

Management of ALS

In the absence of a curative therapy, the management of ALS remains focused on symptom control, with the primary aim of maintaining quality of life (Table 2). Evidence-based management guidelines advise a multidisciplinary model of care, led by a neurologist and clinical nurse consultant working together with physical therapists, occupational therapists, speech pathologists, respiratory physicians, gastroenterologists, psychologists, and social workers to guide patient management. Physical and emotional support should be provided for primary care givers, together with respite care in later stages of ALS. Such an approach has profoundly impacted on patient quality of life and survival [80].

Table 2. Management of symptoms in ALS[a]

Symptom	Treatments
Dyspnea and weak cough	Ventilatory support (NIPPV) Chest physiotherapy and mouth suctioning Coughing techniques Morphine or benzodiazepines (palliative)
Dysphagia	Assessment by speech therapist and dietician Safe swallowing techniques and modified diet Insertion of gastrostomy tube
Weakness and disability	Orthotics Physiotherapy Adaptive aids; for example, wheelchair
Sialorrhea	Anticholinergic medications Botulinum toxin injections Radiation of salivary glands Mouth care products and suction
Thickened saliva	Adequate hydration Saline and *N*-acetylcysteine nebulizers Suctioning Natural remedies (e.g., papaya)
Dysarthria	Speech therapy assessment Communication aids Education of family and caregivers
Pain Musculoskeletal pain and cramps Fasciculations and spasticity Skin pressure pain due to immobility	Physiotherapy Analgesia Repositioning and pressure area care. Pressure-relieving cushions and mattress
Cognitive dysfunction and dementia	Explain symptoms to caregivers and family Antidepressant therapies Respite
Emotional lability	Educate ALS patients and caregivers Pharmacotherapy (antidepressants, benzodiazepines) Dextromethorphan hydrobromide/quinidine sulfate
Depression and anxiety	Counseling Pharmacotherapy (antidepressants, benzodiazepines)
Constipation	Adequate hydration Increase dietary intake of bran, bulk, or fiber Regular oral aperients

[a]In the absence of curative therapies, symptomatic management in a multidisciplinary team setting may prolong survival and quality of life in ALS. Management of respiratory dysfunction by non-invasive positive pressure ventilation (NIPPV) has significantly prolonged survival and improved life quality. NIPPV should be initiated at onset of respiratory symptoms or when respiratory function tests become abnormal, although benefits in early stages of ALS have been reported. Malnutrition and weight loss adversely impact the quality of life and survival in ALS, and should be managed by increasing caloric intake and the initiation of enteral feeding. Implementation of gastrostomy feeding should be discussed early in the disease because significant morbidity may preclude the insertion of a gastrostomy tube in later stages. Importantly, a multitude of additional symptoms develop in ALS and require management (see above). Physical and emotional support should be provided for primary caregivers, together with respite care in the later stages of ALS. In the terminal phase, issues pertaining to anxiety and fear of death, worsening pain, and respiratory dysfunction require management through a multi-disciplinary palliative care model.

Neuroprotection

Although over 30 different therapeutic agents have been investigated in ALS, riluzole has been established as the only effective neuroprotective therapy for ALS, prolonging patient survival by at least 3–6 months, with the beneficial effects of riluzole being most prominent in patients with bulbar-onset disease [74,81]. A potential explanation for therapeutic failure may relate to delay in diagnosis and thereby institution of treatments in later stages of the disease process when these may be less effective.

Cell replacement therapies have become an active area of research in neurodegenerative disorders including ALS [82]. A major challenge posed in ALS relates to the diffuse nature of the disease and the important role non-neuronal cells exert in the pathological process. Additional challenges to this approach include the requirement for extensive surgical intervention to deliver the therapy and concerns regarding the long-term viability and toxicity of transplants.

In humans, stem cells can be classified into embryonic, somatic, and induced pluripotent stem cells [82]. Neuronal stem cells (NSC) may be genetically programmed to develop into neurons or glial cells [83]. Studies in SOD-1 mouse models have established the efficacy of NSC transplantation into the spinal cord [84], with multisite spinal injection being more efficacious [85]. Importantly, neuronal cell based therapies aimed at spinal and supraspinal targets may prove to be more effective [86]. Recent human studies have established the feasibility of NCS approaches, although biological efficacy and long-term safety remain unknown because only a small number of patients have been studied and the follow-up period was short [87].

Efficacy of spinal mesenchymal stem cell transplantation has also been reported in the SOD-1 mouse model, with the transplanted mesenchymal stem cells evolving into astrocytes [88]. Two recent Phase I studies have established the safety of mesenchymal stem cell transplantation approaches in humans [89,90]. Intravenous injection of stem cells resulted in intrathecal localization, raising the prospect of non-surgical delivery methods [90]. In addition, induced pluripotent stem cells have been successfully reprogrammed from fibroblasts derived from hereditary ALS patients and asymptomatic mutation carriers, and differentiated into motor neurons [91]. These stem cell derived models may be utilized in gaining a better understanding of ALS pathophysiology and serve to form platforms for screening novel therapies in ALS [92].

In addition to cell based approaches, strategies aimed at modulating gene expression are emerging as potential novel therapeutic options, particularly in light of significant advances in the understanding of the genetic causes of ALS [82]. One such approach involves the use of antisense oligonucleotides which are short synthetic oligonucleotides (15–25 nucleotides) that bind by Watson–Crick hybridization to target mRNA in a sequence-specific manner. Consequently, the target mRNA is degraded by intranuclear enzymes such as RNase H [93]. The efficacy of antisense oligonucleotide therapy approaches was demonstrated in the SOD-1 animal models [94] wherein intrathecal infusion of antisense oligonucleotides resulted in a reduction of SOD-1 mRNA and protein levels within the CNS, accompanied by clinical improvement [94]. In addition, antisense oligonucleotide therapies were reported to reduce *c9orf72* gene related RNA-toxicity independent of non-ATG translation [30]. Although the animal studies suggested a potential therapeutic benefit of antisense oligonucleotides

Box 2. Outstanding questions

Development of diagnostic biomakers in ALS

At present the diagnosis of ALS remains clinically based, relying on the identification of upper and lower motor neuron features. Consequently, a diagnostic delay occurs, perhaps beyond the therapeutic window period. Combining clinical, neurophysiological, genetic, molecular, and radiological techniques may aid an earlier diagnosis of ALS, with the ultimate aim of recruiting patients into treatment trials earlier in the disease course.

Development of prognostic biomakers in ALS

The assessment of drug efficacy in ALS remains clinically based, relying on measuring the rate of disease progression through the utilization of the ALS rating scale-revised (ALSFRS-R). Such clinical scales may be insensitive, especially in the early stages of the disease process. The development of reliable quantifiable biomarkers remains elusive in ALS, and development of prognostic biomarkers would be crucial for effective evaluation of a therapeutic agent in the early stages of development.

Identification of modifier genes and environmental factors that govern the phenotype and rate of disease progression in ALS

The factors the trigger disease-onset in ALS appear to be different from the factors that mediate disease progression. Co-inheritance of modifier genes, environmental factors, and molecular and autoimmune processes all seem to contribute to the rate of disease progression. Identification of these processes could be therapeutically beneficial.

Determining the pathophysiological mechanisms mediating neurodegeneration in genetic and sporadic forms of ALS

Although 21 genetic mutations and genetic loci have been identified in ALS, the mechanisms by which these genetic mutations lead to neuronal degeneration remain elusive. Identification of these mechanisms may lead to the development of novel therapeutic strategies.

Development of 'good' animal models for ALS

Although several animal models for ALS have been developed, they do not faithfully reproduce the disease as evident in humans. Development of appropriate animal models would be of pathophysiological and therapeutic utility.

The pathophysiological mechanisms underlying ALS appear to be multifactorial, encompassing a complex interplay between molecular and genetic factors

Consequently, future therapeutic directions should probably include a combination of treatment modalities aimed at correcting the underlying pathophysiological process.

therapies, the limitation of such approaches pertains to the methods of drug administration. A recent Phase I study demonstrated safety and tolerability of ISIS 333661 when delivered intrathecally in SOD-1 familial ALS patients [95], providing hope that such highly targeted therapeutic strategies would be feasible in the treatment of ALS.

Strategies aimed at enhancing axonal growth, through inhibition of Nogo (neurite outgrowth inhibitor; RTN4), may also prove therapeutically useful in ALS. Nogo belongs to the reticulon family of proteins which function to inhibit the outgrowth of neurites in the CNS [96]. Three Nogo isoforms have been identified, with the Nogo-A isoform predominating [96]. Importantly, the Nogo-A isoform is upregulated in ALS and appears to be a biomarker for ALS [97]. Anti-Nogo-A antibodies enhance axonal regeneration and improve functional recovery in animal models of acute CNS injury [98]. A randomized controlled Phase I study of the anti-Nogo-A agent Ozanezumab demonstrated good safety and tolerability, with a trend to efficacy [99]. A larger international trial is currently underway to determine the efficacy of this potentially therapeutic approach.

In conclusion, there have been significant advances in the understanding of ALS pathophysiology. Evidence is emerging of a multifactorial process with complex interactions between genetic factors and vital molecular pathways. To date 16 genes and genetic loci have been associated with ALS, and mutations in DNA/RNA-regulating genes, including the recently described *c9orf72* gene, suggest an important role for dysregulation of DNA/RNA and protein metabolism in the pathogenesis of ALS. In addition, dysfunction of vital molecular pathways, including glutamate-mediated excitotoxicity, mitochondrial dysfunction, oxidative stress, and dysregulation of axonal transport, together with abnormalities of non cell autonomous processes, have also been identified as important processes in ALS pathogenesis. These pathophysiological insights have suggested novel therapeutic approaches, including stem cell and genetics-based strategies, providing hope for feasible treatment of ALS. Given the multifactorial nature of the underlying pathophysiological process, combination therapies incorporating stem cell, genomic, and autoimmune (monoclonal) strategies, together with glutamate antagonism, may yet prove useful as an effective strategy in ALS (Box 2).

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