Amyotrophic lateral sclerosis

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Abstract | Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease, is characterized by the degeneration of both upper and lower motor neurons, which leads to muscle weakness and eventual paralysis. Until recently, ALS was classified primarily within the neuromuscular domain, although new imaging and neuropathological data have indicated the involvement of the non-motor neuraxis in disease pathology. In most patients, the mechanisms underlying the development of ALS are poorly understood, although a subset of patients have familial disease and harbour mutations in genes that have various roles in neuronal function. Two possible disease-modifying therapies that can slow disease progression are available for ALS, but patient management is largely mediated by symptomatic therapies, such as the use of muscle relaxants for spasticity and speech therapy for dysarthria.

Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative disease that is characterized by the degeneration of both upper motor neurons (that is, neurons that project from the cortex to the brainstem and the spinal cord) and lower motor neurons (that is, neurons that project from the brainstem or spinal cord to muscle), leading to motor and extra-motor symptoms (FIG. 1). The initial presentation of ALS can vary between patients; some present with spinal-onset disease (that is, the onset of muscle weakness of the limbs), but other patients can present with bulbar-onset disease, which is characterized by dysarthria (difficulty with speech) and dysphagia (difficulty swallowing). In most patients, the cause of ALS is unknown, although some individuals have familial disease, which is associated with mutations in genes that have a wide range of functions, including roles in non-motor cells. In familial ALS, some of the implicated genes are incompletely penetrant, and with rare exceptions, genotype does not necessarily predict phenotype[1]. Although the primary symptoms of ALS are associated with motor dysfunction (such as muscle weakness, spasticity and dysphagia), up to 50% of patients develop cognitive and/or behavioural impairment during the course of disease, and 13% of patients present with concomitant behavioural variant frontotemporal dementia (FTD)[2–4]. The high prevalence of cognitive and/or behavioural symptoms in patients with ALS, coupled with the finding of a hexanucleotide repeat expansion in *C9orf72* (encoding guanine nucleotide exchange C9orf72) as a major cause of ALS and FTD[5,6], have contributed to the recharacterization of ALS as a neurodegenerative rather than a neuromuscular disorder and have signposted the direction of research over the upcoming decade.

The classification of ALS can vary depending on the criteria used. The traditional definitions of ALS subgroups are based on the extent of involvement of upper and lower motor neurons, although other classification systems include different parameters, such as the site of onset (that is, bulbar-onset or spinal-onset disease), the level of certainty of diagnosis according to the revised El Escorial criteria and heritability (sporadic or familial disease)[7]. To date, none of these classification systems have incorporated the cognitive or behavioural symptoms, and within each classification system, a range of sub-phenotypes and clinical trajectories can be observed.

This Primer reviews the aspects of ALS that contribute to disease heterogeneity and looks to the future of new therapeutic trials that incorporate recent advances in our understanding. For new therapies, the challenge is to define mechanisms of disease that are amenable to drug targeting and to define patients who are likely to respond to these therapeutic agents.

Epidemiology

Descriptive epidemiology

Most population-based epidemiological studies of ALS have come from high-quality European patient registers[8]. These population-based registers have been combined to form the European ALS Epidemiology Consortium (EURALS), which has provided data comparing the incidence of ALS between European countries[9]. In Europe, the incidence ranges from 2 to 3 cases per 100,000 individuals. Defined geographical areas are ideally suited to estimate the incidence and prevalence and to support more-detailed studies of risk, clinical trajectory, outcome and utilization of services for ALS[8]. As ALS is

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a rare disease, a population-based approach with multiple sources of ascertainment is the best way to describe the entire phenotypic spectrum[10], as population-based registers provide more complete information about the disease than data sets from specialist clinics, which are often biased in favour of younger patients and those with less-severe disease[10]. Similarly, clinical trial cohorts such as those collected within the US-based Pooled Resource Open-Access ALS Clinical Trials Database (PRO-ACT) data set also select for patients with ALS who have better prognosis; survival within these cohorts is ~12 months longer than survival in population-based cohorts.

Contrary to earlier assumptions, the incidence of ALS differs based on ancestral origin; studies in populations of European origin have shown a crude incidence of >3 cases per 100,000 individuals[11,12], but incidence is lower in east Asia (~0.8 cases per 100,000 individuals) and south Asia (~0.7 cases per 100,000 individuals). In some regions (such as Guam and the Kii peninsula of Japan) the reported incidence was very high but has reduced substantially over the past 30 years for reasons that remain unclear. In areas where different ancestral populations live in close proximity (as in North America), the incidence of ALS in indigenous populations is low (0.63 cases per 100,000 individuals)[13], whereas reported incidences in regions of relatively homogeneous populations (such as Ireland, Scotland and the Faroe Islands) are high (2.6 cases per 100,000 individuals)[9,14].

In addition, variations in the phenotype and natural history of ALS have been reported in different ancestral populations; indeed, reported survival is shorter in Europe (24 months) than in central Asia (48 months)[15]. In addition, admixed populations (that is, populations of mixed ancestry) might have lower mortality from ALS. In a population-based study in Cuba, the mortality rate of patients was ALS was 0.55 per 100,000 individuals in an admixed population but was ~0.9 per 100,000 individuals in white or black individuals[16], confirming the importance of ancestral origin in disease risk. In Europe, most men have spinal-onset disease, and women have a higher propensity for bulbar-onset disease[9]. The percentage of individuals with bulbar-onset disease is much lower in Asia than in Europe, but a north-to-south gradient has been reported in Europe, with a higher percentage of individuals with spinal-onset disease in southern Europe[9]. On the basis of available data, the age of diagnosis and first symptoms is higher in Europe than in Asia and South America. In Europe, the age of onset peaks at 65 years[9]. The main limitation of global ALS epidemiology is that ~80% of studies have been conducted in Europe and the United States, and they mainly comprise patient cohorts of northern European ancestry. International consortia collecting data in areas with admixed populations and in different continents will be required to fully elucidate the range of clinical presentations and to understand the roles of ancestry, genetics and environmental exposures in ALS causation.

Causes of ALS

Genetics. ALS is considered a complex genetic disorder with a Mendelian pattern of inheritance in some cases but with no discernible family history in the rest. Mathematical models developed using population-based registers have suggested that individuals with ALS are likely to carry a number of 'at risk' variants that interact with environmental factors through a series of at least six notional steps leading to disease manifestation. One of these steps is thought to be the genetic risk (from birth), but the interplay of environmental factors that lead to the remaining steps has yet to be defined. In transgenic mice, the genetic background can alter the phenotypic presentation of ALS[17,18], suggesting that human disease phenotypes also have a genetic basis and that genomic and epigenomic 'fingerprinting' could permit the clustering of different phenotypic manifestations into discrete underlying causes that are amenable to therapeutic intervention.

Large, combined genome-wide association studies (GWAS) of seemingly sporadic ALS suggest that the genetic architecture is based primarily on rare variants, in contrast to other diseases that are associated with large numbers of common variants, such as schizophrenia. GWAS in ALS are also complicated because the rare variants that confer risk might be specific to individuals, families and ancestral populations[19], rendering GWAS less suited for the study of ALS genetics than for schizophrenia. Initiatives such as the Project MinE Consortium (www.projectmine.com), which aims to undertake whole-genome sequencing of >15,000 patients with ALS and >7,500 control individuals, are expected to provide better clarity of the genetic architecture of ALS.

Of the known genes that have a major effect on the development of ALS (TABLE 1), our current knowledge comes primarily from the study of ancestral European (Europe, Canada, Australia and the United States) and east Asian populations; within these populations, the dichotomization of ALS into 'familial' and 'sporadic' subtypes is an over-simplification. Although ≥30 genes confer a major risk of ALS, evidence suggests roles of oligogenic inheritance (in which a phenotypic trait

is determined by more than one gene) and of genetic pleiotropy (in which a single gene has multiple phenotypic manifestations). Within populations of European descent, up to 20% of individuals with ALS have a family history of either ALS or FTD (familial ALS), and of these, four genes account for up to 70% of all cases of familial ALS, namely, *C9orf72*, *TARDBP* (encoding TAR DNA-binding protein 43, TDP43), *SOD1* (encoding superoxide dismutase) and *FUS* (encoding RNA-binding protein FUS)[20]. However, even in the case of these known Mendelian-inherited genes, familial forms of ALS are often characterized by <50% penetrance and genetic pleiotropy, with evidence of oligogenic and polygenic inheritance in individuals with seemingly sporadic disease[21,22].

Environmental and lifestyle factors. Epidemiological case–control studies have aimed to determine the environmental causes of ALS. Early epidemiological studies from regions with a high incidence of ALS and dementia such as Guam and the Kii peninsula of Japan suggested a role for neurotoxins contained within cycad seeds, including β-methylamino-L-alanine. Although the role of β-methylamino-L-alanine in the risk of ALS has not been substantiated[23], a possible role for related cyanotoxins has been proposed, and exposure to water containing cyanobacterial blooms has been suggested to contribute to the risk of ALS in susceptible individuals[24].

ALS has been reported at a higher frequency among groups of athletes than in the general population, although whether physical activity is a risk factor for ALS or is simply a marker of underlying athletic prowess is unclear. Data from a study in the United Kingdom suggested that individuals with ALS had higher rates of pre-morbid physical activity, but two European studies suggested either no effect or a protective effect[22–24]. Reasons for this discrepancy might be due to study design and true population-based differences. However, because ALS is a rare disease, smaller case–control studies are often underpowered and are subject to both bias and error in interpretation. To address these problems in study design, a very large case–control study has been completed as part of the Euro-MOTOR project (www.euromotorproject.eu), which has collected data from >1,500 population-based incident cases and 3,000 matched controls across three countries. Analysis is ongoing, although preliminary data suggest that exposure to smoking might increase the risk of developing ALS, but type 2 diabetes mellitus, high levels of circulating lipids and exposure to female contraceptive hormones might be protective[25,26].

Mechanisms/pathophysiology

Histopathology

Although the fundamental pathophysiological mechanisms underlying ALS are not well understood, the neuropathological hallmark of disease is the aggregation and accumulation of ubiquitylated proteinaceous inclusions in motor neurons. Protein inclusions occur in other neurodegenerative disorders (such as amyloid plaques in Alzheimer disease and synuclein-containing Lewy bodies in Parkinson disease). The biological processes leading to formation of these inclusions has been intensively researched but is poorly understood[4].

In most subtypes of ALS, TDP43 is the main constituent of these inclusions, although mutations in *TARDBP* are a rare cause of ALS[27,28]. Indeed, ~97% of patients with ALS have features of a TDP43 proteinopathy, with depletion of TDP43 in the nucleus but the formation of cytoplasmic aggregates with skein-like or compact morphology in residual motor neurons (FIG. 2a). In specific subtypes of ALS, other types of protein aggregates might be observed, such as neurofilamentous hyaline conglomerate inclusions (FIG. 2b) and the accumulation of misfolded SOD1 in patients with *SOD1*-associated ALS and sequestosome 1 (also known as P62, encoded by *SQSTM1*)-positive, TDP43-negative inclusions that are caused by dipeptide repeat proteins and might be observed outside the motor system in patients with ALS associated with *C9orf72* mutations (FIG. 2c). Although protein aggregates are the hallmark of ALS, the high-molecular-weight complexes that precede the formation of the aggregates, rather than the aggregates themselves[29,30], might be the toxic species. Shedding of higher-molecular-weight protein complexes might mediate cell-to-cell propagation of disease, linking the progression of ALS to a prion-like mechanism, as has also been suggested for diseases mediated by tau and synuclein[31,32].

The gross pathological features of ALS comprise skeletal muscle atrophy, atrophy of the motor cortex and pallor and sclerosis of the pyramidal tracts (that is,

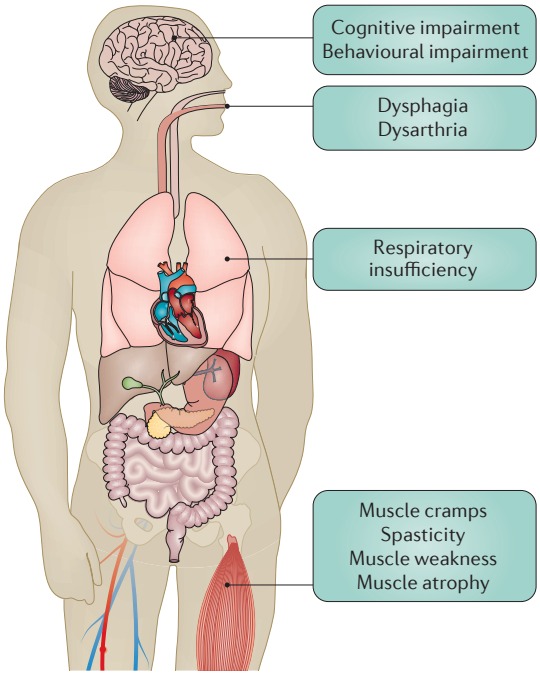

Figure 1 | **Clinical manifestations of ALS.** Although motor manifestations such as muscle weakness and dysphagia (difficulty swallowing) are the main clinical manifestations of amyotrophic lateral sclerosis (ALS), up to half of patients have non-motor symptoms, such as cognitive impairment. Dysarthria; difficulty with speech.

the corticospinal and corticobulbar tracts), together with thinning of the hypoglossal nerves (which are involved in the control of the muscles of the tongue) and the ventral roots of the spinal cord. Microscopic examination usually reveals a depletion of ≥50% of spinal motor neurons and diffuse astrocytic gliosis and microglial infiltration in the grey and white matter of the spinal cord (FIG. 2d–f). Axonal loss, gliosis and myelin pallor are observed in the corticospinal tracts, and astrocytic gliosis is usually observed in the motor cortex, together with a variable depletion of upper motor neurons. Skeletal muscle shows features of denervation and re-innervation, with fibre type grouping and clusters of angular atrophic fibres.

Overview of pathophysiology

Progress has been made in the identification of the genetic causes of ALS[21,22], and models in rats, mice, zebrafish, flies, worms and yeast have been developed to study how mutations cause motor neuron degeneration and to model biological processes that are thought to be important in disease pathobiology. All of these models have limitations, and none fully recapitulates human disease, which is partly because most models are based on gene overexpression (with multiple copies of the human variant inserted into the transgenic model) and because the human neuraxis differs substantially from that of lower animals. Nevertheless, findings from animal models can contribute to our understanding of the cell biology underlying neurodegeneration and can open new avenues towards targeted drug development. In reality, the cellular disruption in ALS is likely the result of many different interacting mechanisms that culminate in larger network disruption, and the separation of different mechanisms is somewhat artificial. This is exemplified by the finding that multiple factors can contribute to neuronal damage in models with *SOD1* mutations (BOX 1). The relative extent to which each of these factors contributes to the overall pathobiology of human disease cannot be fully ascertained, and it would be erroneous to assume that all of these factors are involved in all cases of ALS, as human disease is heterogeneous. Nonetheless, each of the thematic areas should be considered in detail, as they represent our current knowledge base of the pathophysiology of ALS and are the drivers of current and future therapeutic initiatives (FIG. 3).

Table 1 | **Main genes implicated in amyotrophic lateral sclerosis**

Locus	Gene (protein)	Inheritance	Implicated disease mechanisms	Refs
ALS1	*SOD1* (superoxide dismutase 1)	AD or AR	Oxidative stress	234,235
ALS2	*ALS2* (alsin)	AR	Endosomal trafficking	236,237
ALS3	Unknown	AD	Unknown	238
ALS4	*SETX* (senataxin)	AD	RNA metabolism	239
ALS5	Unknown	AR	DNA damage repair and axon growth	240
ALS6	*FUS* (RNA-binding protein FUS)	AD or AR	RNA metabolism	241,242
ALS7	Unknown	AD	Unknown	243
ALS8	*VAPB* (vesicle-associated membrane protein-associated protein B/C)	AD	Endoplasmic reticulum stress	42
ALS9	*ANG* (angiogenin)	AD	RNA metabolism	244
ALS10	*TARDBP* (TAR DNA-binding protein 43)	AD	RNA metabolism	27,245
ALS11	*FIG4* (polyphosphoinositide phosphatase)	AD	Endosomal trafficking	246
ALS12	*OPTN* (optineurin)	AD or AR	Autophagy	247
ALS13	*ATXN2* (ataxin 2)	AD	RNA metabolism	248
ALS14	*VCP* (valosin-containing protein)	AD	Autophagy	36
ALS15	*UBQLN2* (ubiquilin-2)	XD	UPS and autophagy	34
ALS16	*SIGMAR1* (sigma non-opioid intracellular receptor 1)	AD	UPS and autophagy	249,250
ALS17	*CHMP2B* (charged multivesicular body protein 2B)	AD	Endosomal trafficking	251
ALS18	*PFN1* (profilin 1)	AD	Cytoskeleton	97
ALS19	*ERBB4* (receptor tyrosine-protein kinase erbB 4)	AD	Neuronal development	252
ALS20	*HNRNPA1* (heterogeneous nuclear ribonucleoprotein A1)	AD	RNA metabolism	82
ALS21	*MATR3* (matrin 3)	AD	RNA metabolism	83
ALS22	*TUBA4A* (tubulin α4A)	AD	Cytoskeleton	102
ALS-FTD1	*C9orf72* (guanine nucleotide exchange C9orf72)	AD	RNA metabolism and autophagy	5,6
ALS-FTD2	*CHCHD10* (coiled-coil-helix-coiled-coil-helix domain-containing 10)	AD	Mitochondrial maintenance	253
ALS-FTD3	*SQSTM1* (sequestosome 1)	AD	Autophagy	254
ALS-FTD4	*TBK1* (serine/threonine-protein kinase TBK1)	Unknown	Autophagy	53,54

AD, autosomal dominant; AR, autosomal recessive; UPS, ubiquitin–proteasome system; XD, X-linked dominant

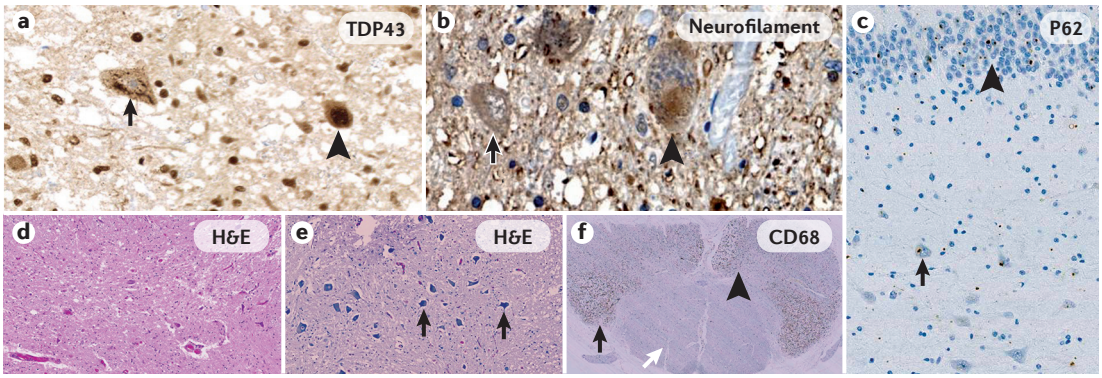

Figure 2 | **Histopathology of ALS. a** | Normal localization of TAR DNA-binding protein 43 (TDP43) in the nucleus (arrowhead) and aberrant localization in a diseased neuron with loss of nuclear expression and a 'skein-like' inclusion in the cytoplasm (black arrow). **b** | Normal motor neuron (black arrow) and a hyaline conglomerate inclusion that is labelled for neurofilament (arrowhead) in a patient with amyotrophic lateral sclerosis (ALS) caused by a *SOD1* mutation. **c** | P62-positive, TDP43-negative dipeptide repeat inclusions with a 'stellate' morphology in the pyramidal cells of CA4 (black arrow) and granule cells of the dentate fascia (arrowhead) in the hippocampus of a patient with ALS caused by a mutation in *C9orf72*. **d** | Depleted numbers of motor neurons (the absence of arrows) in the ventral horn of the spinal cord in a patient with ALS. **e** | Motor neurons (arrows) in the spinal cord ventral horn of a healthy individual. **f** | Marked microglial reactivity (CD68 labelling) in the lateral tracts (black arrow) and ventral horns (arrowhead), with no labelling in the dorsal columns (white arrow) of the spinal cord in a patient with ALS. H&E, haematoxylin and eosin.

Impaired protein homeostasis

Mutations in some genes leads to the translation of proteins that are misfolded, that have an abnormal cellular localization or are aberrantly formed and that can directly or indirectly impair the proteasomal or autophagic machinery of the cell, leading to impaired protein turnover. Indeed, genes associated with familial ALS encode proteins that can promote dysfunction of the ubiquitin–proteasome system. For example, mutant SOD1 is associated with reduced expression of components of the ubiquitin–proteasome system[33] and transitional endoplasmic reticulum ATPase (also known as valosin-containing protein, or VCP) and ubiquilin-2 (encoded by *UBQLN2*) have a role in substrate delivery to the proteasome, which is disrupted in the presence of ALS-associated mutations[34–36]. In addition, dysregulation of chaperone proteins has been identified in ALS associated with *SOD1* and *TARDBP* mutations[37–40]. Mutations in *VAPB* (encoding vesicle-associated membrane protein-associated protein B/C, also known as VAPB) can cause defective activation of the unfolded protein response in disease models[41,42].

C9orf72 is a key regulator of autophagy initiation[43], and loss of this function might contribute to the presence of ubiquitin-positive, P62-positive and TDP43-negative inclusions in extra-motor areas of the central nervous system (CNS) in ALS associated with mutations in *C9orf72*. P62, optineurin (encoded by *OPTN*) and ubiquilin-2 have a role in the early steps of autophagy[44–46], and alsin, polyphosphoinositide phosphatase (also known as FIG4), VCP and charged multivesicular body protein 2b are involved in the maturation of autophagosomes into autophagolysosomes by regulating the fusion of autophagosomes with multivesicular bodies, endosomes and lysosomes[47–51]. Mutations in *SQSTM1* might disrupt the delivery of autophagic substrates to the autophagosome[52], and mutations in *UBQLN2* and *OPTN* are also associated with ALS. The activities of P62 and optineurin are regulated by serine/threonine-protein kinase TBK1[53,54], and haploinsufficiency of *TBK1* is a cause of familial ALS, which supports the hypothesis that reduced substrate delivery to autophagosomes might contribute to motor neuron injury in ALS. Reduced VCP activity due to *VCP* mutations has been shown to decrease the maturation of autophagosomes. Other proteins implicated in ALS pathophysiology, including mutant alsin and FIG4, can affect autophagy at the stage of initiation, although the mechanism for this effect is unclear[47,55]. Both SOD1 and TDP43 are known substrates of autophagy, suggesting that defective autophagy contributes to the toxic accumulation of these proteins in ALS. The formation of dipeptide repeat proteins through repeat-associated non-ATG translation from the expanded RNA repeat of *C9orf72* might also result in dysproteostasis, but this remains to be conclusively demonstrated and the mechanism elucidated (see Nucleocytoplasmic and endosomal transport).

Aberrant RNA metabolism

Alteration of mRNA processing is a key theme in ALS pathogenesis[56]. mRNA undergoes complex processing as it transits from the nucleus to cytoplasm, where it is translated. In neurons, mRNA can be transported to allow local protein translation in the axonal compartment. Although the functional consequences of RNA dysregulation that lead to age-related and selective degeneration of neuronal populations remain poorly understood, analysis of the translatome of actively transcribed mRNAs will be essential in elucidating the upstream molecular events that contribute to neuronal injury.

The discovery of mutations in *TARDBP* and *FUS* as rare causes of ALS has identified a crucial pathogenetic role for RNA-binding proteins that contain low-complexity domains[57]. Mutant TDP43 or FUS mislocalize

Box 1 | **Mechanisms of SOD1 toxicity in cellular and rodent models**

Transgenic mice harbouring mutations in *SOD1* (encoding superoxide dismutase) can be used to study amyotrophic lateral sclerosis (ALS) pathophysiology. These mice have overexpression of mutant SOD1 and many have an aggressive disease course over ~80–90 days. However, they have clinical and pathological features that are similar to human ALS. *SOD1* mutations can lead to neurotoxicity in several ways, including protein misfolding, proteasome impairment, excitotoxicity, oxidative stress, endoplasmic reticulum stress, impaired axonal transport, axonopathy, inflammation, altered RNA processing and mitochondrial dysfunction[231]. Other mechanisms of SOD1-related neurotoxicity have recently emerged and have gained interest. SOD1 can be a transcription factor for genes involved in resistance to oxidative stress and repair of oxidative damage[232]. Indeed, RNA oxidation is emerging as a prominent pathological outcome of generalized oxidative stress in the cell, with an increasing importance in neurodegeneration. Astrocytes and oligodendrocytes reprogrammed from fibroblasts of patients with *SOD1* mutations have been shown to induce hyperexcitability and cell death in healthy control motor neurons. Glial toxicity is mediated through both contact (lactate-independent) and soluble mechanisms, and is rescued by *SOD1* knockdown using short hairpin RNA in glia from patients with *SOD1*-related ALS but also in glia derived from patients with sporadic ALS without *SOD1* mutations[112]. Wild-type and mutant SOD1 form insoluble intraneuronal fibrils, which aggregate with increased propensity in the mutant form. Prion-like transmission of mutant SOD1 fibrils can seed the aggregation of wild-type SOD1 in neighbouring neurons and can propagate neuronal injury[233].

from the nuclear to the cytoplasmic compartment, which is hypothesized to result in the loss of the normal processing of their target RNAs[58,59]. Indeed, up to one-third of the transcriptome is altered in models of *TARDBP*-related ALS[60], and dysregulation of gene expression has also been observed in relation to mutations in *C9orf72*, *SOD1* and *FUS*[61], including changes in transcription, alternative splicing of mRNA, axonal transport of mRNAs and biogenesis of microRNAs[62,63].

The GGGGCC repeat expansion in the non-coding region of *C9orf72* can cause ALS and leads to the formation of stable parallel unimeric and multimeric G-quadruplex structures, which avidly interact with RNA processing factors[64,65]. In addition, the repeat expansion leads to the production of abnormal RNA species that can be identified as nuclear RNA foci, and might induce direct RNA toxicity by, for example, sequestering RNA-binding proteins[66–68]. Indeed, numerous proteins that bind to the repeat expansion have been identified[69]. In addition, repeat expansions in *C9orf72* could lead to the formation of R-loops (that is, DNA–RNA hybrid structures) that increase susceptibility to DNA damage and genome instability[70,71]. Indeed, R-loops and genome instability due to double-strand DNA breaks and defective serine-protein kinase ATM-mediated DNA repair are important components of neuronal injury due to a GGGGCC repeat expansion in *C9orf72* (REF. 72).

Mutations in *ANG* (encoding angiogenin, which has a role in RNA processing[73,74]) and *SETX* (encoding senataxin, which regulates the transcription of ribosomal RNA[75,76]) are associated with ALS and might lead to disturbances in RNA metabolism. In addition, mutations in *ELP3* (encoding elongator complex protein 3), *TAF15* (encoding TATA-binding protein-associated factor 2N) and *EWSR1* (encoding RNA-binding protein EWS)[77–79] have also been associated with ALS. These genes encode proteins that are involved in the regulation of RNA metabolism; ELP3 contributes to the regulation of transcription elongation, and TAF15 and EWS, which are functionally and structurally related to FUS, have a role in the control of transcription and alternative splicing[80,81]. Mutations in other genes involved in RNA metabolism, such as *HNRNPA1*, *HNRNPA2B1* and *MATR3*, have been implicated in ALS[82,83]. The mislocalization of the mutant proteins into the cytoplasm might result in a toxic gain of function, and the effect of these proteins on the formation of stress granules is an area of intense research[84–86].

Nucleocytoplasmic and endosomal transport

In addition to altering RNA metabolism, the GGGGCC repeat expansion in *C9orf72* is believed to affect the intracellular localization of *C9orf72* mRNA and can lead to the production of dipeptide repeat proteins. For example, increased binding of mRNA export adaptors to expanded *C9orf72* pre-mRNAs might target the pre-mRNAs for nuclear export, which could enable the translation and production of abnormal dipeptide repeat protein species[68,87]. Indeed, sequestration of the nuclear export adaptor serine/arginine-rich splicing factor 1 (SRSF1) by the repeat expansion region of *C9orf72* RNA, triggers nuclear RNA export factor 1 (NXF1)-dependent nuclear export of *C9orf72* transcripts retaining the hexanucleotide repeats, allowing repeat-associated non-ATG translation of these transcripts into dipeptide repeat proteins. Depletion of SRSF1 in cellular and *in vivo* models reduces the production of dipeptide repeat proteins and neurotoxicity[88]. Dipeptide repeat proteins interfere with proper nucleocytoplasmic transport and lead to neurotoxicity by several mechanisms[89,90]. For example, liquid-phase separation of RNA-binding proteins has a role in the production of membraneless organelles involved in RNA processing; arginine-rich dipeptide repeat proteins isolated from *C9orf72* expansions can induce phase separation of proteins that have a role in RNA and stress granule metabolism, thereby producing spontaneous stress granule assembly[91].

Endosomal and vesicle transport

TDP43 has a role in the regulation of endosomal trafficking, and loss of TDP43 function has been shown to alter dendritic endosomes, which results in reduced neuronal health[92]. Other ALS-associated gene variants, such as mutations in *ALS2* and *UNC13A* (encoding protein UNC-13 homologue A) variants can also affect endosomal and vesicle transport. Indeed, the gene product of *ALS2,* alsin, is a guanine nucleotide-exchange factor for the small GTPase Rab5 and is involved in endosome trafficking and fusion[55,93], and UNC-13A is involved in priming synaptic vesicles and in neurotransmitter release[94].

Axon structure and function

The identification of mutations in *DCTN*, *PFN1* and *TUBA4A* in patients with ALS suggests that abnormalities in proteins that are essential for axonal transport are involved in ALS pathophysiology[95–97]. In addition, mutations in *NEFH* (encoding neurofilament heavy polypeptide) have been described in a small number of patients[98],

although whether these mutations are pathogenetic through axonal dysfunction remains to be observed. Rare mutations in *PRPH* might have a role in ALS pathogenesis, due to effects on neurofilament housekeeping functions including protein cargo trafficking[99,100].

DNA repair

Impaired DNA repair was suggested to have a role in ALS pathophysiology following the identification of *FUS* mutations, although the exact role of DNA repair failure in ALS remains to be clarified[101,102]. Mutations in *NEK1* and *C21orf2*, both of which encode proteins involved in DNA repair, can cause ALS[19,103,104], although the biological pathways associated with their causal role await confirmation.

Excitotoxicity

Motor neurons are very sensitive to toxicity induced by calcium entry following excessive glutamate stimulation, as they have a lower calcium buffering capacity and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that are more calcium permeable (as they contain less of the GluR2 subunit) than other neuronal subtypes[105]. In addition, the excitatory amino acid transporter 2 (EAAT2), an astroglial protein that is the main synaptic glutamate reuptake transporter, is impaired in ALS, which is likely to result in synaptic glutamate abundance and motor neuron toxicity. The loss of EAAT2 has been observed in both rodent models and patients with ALS. Excitotoxicity is thought to be a mechanism common to all forms of ALS, although the

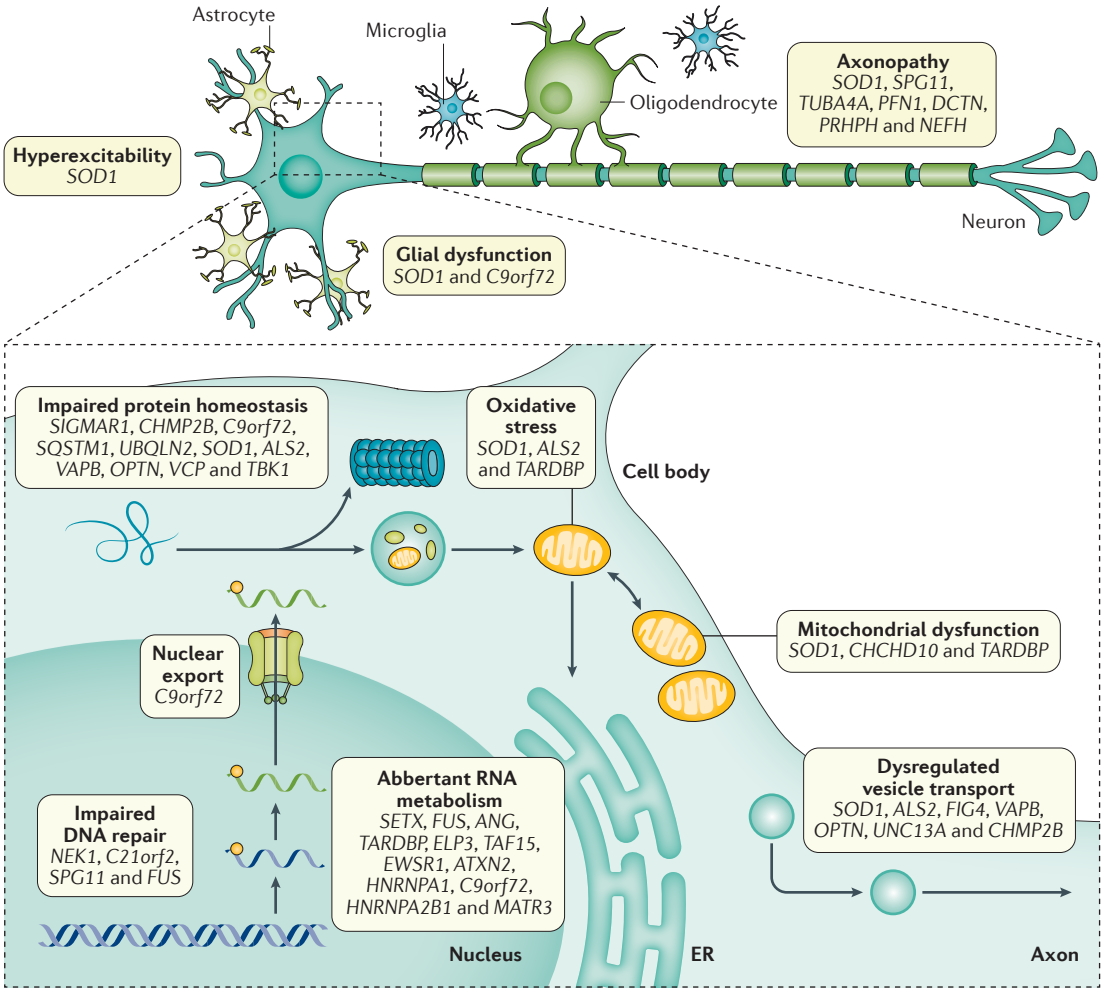

Figure 3 | **Pathophysiology of ALS.** Mutations in several genes that have been implicated in the pathophysiology of amyotrophic lateral sclerosis (ALS) can exert motor neuronal injury through more than one pathophysiological mechanism, although these mechanisms are often interlinked. *SOD1* is the longest-studied gene implicated in ALS and has been linked to the greatest number of pathophysiological mechanisms, whereas the effects of some mutations, such as those in *ALS3* and *ALS7*, are still unknown. Aberrant RNA metabolism and impaired protein homeostasis are predominant factors linking multiple ALS causative genes to neuronal injury. Mitochondrial dysfunction can arise from a mutation in *CHCHD10* and from secondary respiratory chain deficiencies that arise from protein aggregates generated in the presence of other ALS-associated mutations. Both cases lead to an increase in oxidative stress, which puts further stress on an already impaired protein homeostasis system. Other mechanisms of ALS can directly alter neuronal function (such as impaired nuclear export, impaired DNA repair and dysregulated vesicle transport) and dysfunction of glial cells. In addition, neuronal hyperexcitability and axon dysfunction have been implicated in ALS. The interplay of mechanisms is indicated by arrows. ER, endoplasmic reticulum.

evidence for this remains indirect. For example, riluzole, which can attenuate disease progression and is approved by the US FDA for ALS, can inhibit glutamate release[106,107], although whether this mechanism underlies the therapeutic effect is unclear.

Oligodendrocyte degeneration

Oligodendrocyte degeneration has been observed in ALS. In the healthy CNS, oligodendrocytes are replaced by the proliferation of oligodendrocyte precursor cells, which are abundantly present[108,109]. At least in animal models of ALS, and for reasons that are not clear, oligodendrocyte precursor cells fail to undergo the final stages of differentiation. Oligodendrocytes provide vital metabolic support to axons through the transport of lactate through monocarboxylate transporter 2 (REFS 110,111) and, accordingly, dysfunction of oligodendrocytes contributes to the motor neuron axonopathy in ALS. Restoring oligodendrocyte function by deleting mutant *SOD1* significantly slows disease progression and prolongs the lifespan of mice[112]. Patients with ALS can have abnormalities in oligodendrocytes, but whether these changes contribute to the disease pathogenesis remains to be demonstrated.

Neuroinflammation

Neuroinflammation can be observed in imaging studies in patients with ALS, human post-mortem samples and rodent models of ALS[113,114]. Astrocytes and microglial cells release a number of hazardous and possibly neuroprotective factors. Deleting mutant *SOD1* from these cells in a mouse model increases survival and slows disease progression[115], indicating that inflammation is an important mechanism for neuronal injury and ALS progression. Microglia have dual activation phenotypes, which can be neuroprotective (the M2 phenotype) or toxic (the classically activated or M1 phenotype); data from *SOD1*-transgenic mice suggests that the phenotype of microglia evolves from a neuroprotective phenotype at disease onset to a neurotoxic phenotype, with an altered cytokine release profile, at end-stage disease[116]. In addition, complex signalling between CNS resident immune cells and peripheral cells, including monocytes and T cells has been reported.

Mitochondrial dysfunction

Mitochondrial function is impaired in ALS, and changes in mitochondrial morphology have been observed in some patients and in mice harbouring mutations in *SOD1* (REFS 117,118). In these mice, vacuoles containing protein aggregates that include mutant SOD1 can be observed in the mitochondrial inter-membrane space, leading to impairments in protein import[119]. In addition, oxidative damage to mitochondrial proteins that leads to defects in respiratory chain function has been observed in patients with ALS and in mice harbouring mutations in *SOD1* (REF. 120), and several experimental models of ALS have defects in the axonal transport of mitochondria, which could contribute to the axonopathy at the neuromuscular junction[121,122].

Many of the cellular functions that are disrupted in ALS are regulated by signalling between the endoplasmic reticulum and mitochondria, underpinned by associations mediated by the endoplasmic reticulum protein VAPB and the outer mitochondrial protein regulator of microtubule dynamics protein 3 (REF. 123). These associations are perturbed by *TARDBP* and *FUS* mutations[124,125]. TDP43 preferentially binds to mRNAs encoding respiratory chain complex 1 subunits, causes complex 1 disassembly[126] and accumulates in the mitochondria of patients with ALS. Moreover, mutations in *TARDBP* increase the mitochondrial localization of TDP43. Suppressing the localization of TDP43 to mitochondria improves mitochondrial function and reduces neuronal loss associated with mutant TDP43 in cell-based models. In models of *C9orf72*-associated ALS, the dipeptide repeat protein poly(GR) seems to compromise mitochondrial function, cause oxidative stress and cause DNA damage[127]. *CHCHD10* mutations, which are associated with familial ALS, can promote the loss of mitochondrial cristae junctions, impair mitochondrial genome maintenance and interfere with apoptosis by preventing cytochrome *c* release[128].

Final common pathway

The main mechanism that is involved in the pathogenesis of ALS is probably dependent on the initial cause of disease, although multiple mechanisms seem to explain the toxicity of each mutation, and these mechanisms are probably interlinked. This association is clearly the case for *SOD1* mutations. With regards to *C9orf72* repeat expansions, several factors probably contribute to neuronal injury, including toxic gains of function due to RNA foci and the presence of dipeptide repeat proteins, but loss of the normal function of C9orf72 might also have a role.

Whatever the underlying mechanisms of ALS, the end result is that the motor neuron cannot maintain its axonal projections, leading to axonal retraction and denervation of the target cell. For lower motor neurons, this axon retraction results in denervation of the muscle, but for upper motor neurons, this retraction results in the loss of proper control of lower motor neurons, hypertonicity and weakness. In addition, a loss of important neural networks within motor domains and extra-motor domains is apparent[129]. As many of the proteins encoded by genes that are implicated in ALS are ubiquitously expressed (TABLE 1), it is unclear why motor neurons are the most susceptible cell type to the hazardous effects of these mutations. The large size of motor neurons, and the need to maintain their long axonal projections, could make these cells more sensitive to metabolic abnormalities than others, but other neuronal subtypes, such as sensory neurons, have even longer axonal projections. Other factors that might have a role are the high expression of ephrin type-A receptor 4 and matrix metalloproteinase 9 and the low expression of osteopontin and insulin-like growth factor 2 by motor neurons, which might limit axonal sprouting and repair. Of particular interest is that within the motor neuron pool, neurons that establish the fast-fatiguable motor units die first in ALS[130,131], but how this relates to the other vulnerability factors needs to be clarified.

Diagnosis, screening and prevention

Clinical presentations

The clinical hallmark of ALS is the involvement of both upper and lower motor neurons (FIG. 1). Patients can present with symptoms of predominantly upper motor neuron dysfunction (that is, spasticity and weakness), with the involvement of lower motor neurons only becoming evident at later stages of disease[7,132–135]. Conversely, patients can present with symptoms of lower motor neuron dysfunction, which includes fasciculations, cramps and muscle wasting. Approximately one-third of patients with ALS present with bulbar-onset disease, which is characterized by progressive dysarthria, followed by dysphagia and often with associated emotional lability. Limb-onset disease accounts for 60% of cases, is usually asymmetrical in presentation and can first develop in the upper or lower limbs. Up to 5% of patients present with respiratory problems, and these patients are often observed in cardiology and pulmonology clinics before they are referred to neurology clinics[136]. In these cases, patients can also present with unexplained weight loss. Data suggest that some patients with ALS are hypermetabolic[137], although the pathophysiology underlying this symptom is not well understood. Cardiovascular risk factors (such as hyperlipidaemia or obesity) seem to be protective[137] but do not alter clinical outcome[138]. Patients can present with a pure motor phenotype of ALS and have normal cognition and behaviour, but some patients can present with a purely cognitive or behavioural phenotype consistent with FTD or with a mixed phenotype with minor changes in executive impairment that progress over time. FTD is one of the presenting features in 13% of incident cases[2–4], and ~30% of all newly-diagnosed patients have some evidence of executive dysfunction at presentation[3,139]. Depending on the population and the extent of cognitive testing performed, most studies have suggested that up to 50% of patients do not have cognitive impairment throughout the course of the disease[3]. Behavioural changes are common in patients with ALS, with apathy as the most prevalent symptom. Detailed assessments of behavioural changes in patients with ALS using a disease-specific behavioural scale (that is, the Beaumont Behavioural Inventory) have suggested that up to 40% of patients with newly-diagnosed ALS have behavioural changes that can be clustered into at least five different groups that roughly map to known neuroanatomical networks and pathways[140]. Substantial autonomic impairment (such as cardiovascular, gastrointestinal and bladder dysfunction) does not occur in most patients with ALS.

Box 2 | **El Escorial and Airlie House criteria for the diagnosis of ALS[141]**

The presence of:
(a) Evidence of lower motor neuron degeneration by clinical, electrophysiological or neuropathological examination;
(b) Evidence of upper motor neuron degeneration by clinical examination; and
(c) Progression of the motor syndrome within a region or to other regions, as determined by history or examination; and,

The absence of:
(a) Electrophysiological and pathological evidence of other disease processes that might explain the signs of lower or upper motor neuron degeneration; and,
(b) Neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

Categories of Diagnostic Certainty (El Escorial criteria)
Definite ALS: Upper and lower motor neuron signs in three regions.
Probable ALS: Upper and lower motor neuron signs in at least two regions, with upper motor neuron signs rostral to (above) lower motor neuron signs.
Possible ALS: Upper and lower motor neuron signs in one region, upper motor neuron signs alone in two or more regions, or lower motor neuron signs above upper motor neuron signs.
Suspected ALS:* Lower motor neuron signs in only two or more regions.

Categories of Diagnostic Certainty (Airlie House criteria)
Clinically definite ALS: clinical evidence alone of upper and lower motor neuron signs in three regions.
Clinically probable ALS: clinical evidence alone of upper and lower motor neuron signs in at least two regions with some upper motor neuron signs rostral to the lower motor neuron signs.
Clinically probable–laboratory-supported ALS: clinical signs of upper and lower motor neuron dysfunction in only one region, or upper motor neuron signs alone in one region with lower motor neuron signs defined by electromyography criteria in at least two limbs, together with proper application of neuroimaging and clinical laboratory protocols to exclude other causes.
Possible ALS: clinical signs of upper and lower motor neuron dysfunction in only one region, or upper motor neuron signs alone in two or more regions; or lower motor neuron signs rostral to upper motor neuron signs and the diagnosis of clinically probable–laboratory-supported ALS cannot be proven.

*This category has been deleted from the revised El Escorial criteria.

Diagnostic criteria

No definitive test for the diagnosis of ALS is available, and diagnosis is a process of clinical investigation to exclude other possible causes of the presenting symptoms and requires evidence of disease progression. However, the growing understanding of the extra-motor features of ALS, the presence of phenotypic overlap with other neurodegenerative diseases and the identification of genetic and pathological subtypes of ALS can confound accurate and timely diagnosis[7].

Diagnosing ALS is based on the El Escorial and Airlie House criteria[141] (BOX 2). Diagnosis according to these criteria requires a history of progressive weakness spreading within a region or to other regions, such as bulbar regions (affecting speech and swallowing), cervical regions (affecting the upper limbs), thoracic regions (affecting the chest wall and abdominal muscles) or lumbar regions (affecting the lower limbs), with evidence of the involvement of lower motor neurons (through the presence of specific symptoms or evidence of denervation on electromyography) and upper motor neurons (through the presence of specific symptoms and brisk deep tendon reflexes). In the original El Escorial criteria, diagnostic certainty ranged from suspected ALS (although this is no longer included in the revised criteria) to definite ALS (in individuals with mixed upper motor neuron and lower motor neuron findings in three body regions), which relates to the burden of disease. In the Airlie House criteria, diagnostic certainty ranges from clinically definite ALS to possible ALS. Neurophysiological findings have been classified using the Awaji criteria, which can enhance diagnostic and prognostic sensitivity[142]. Variants of the El Escorial criteria are used in research settings and for clinical trial enrolment, but these criteria should not be

used in clinical practice for routine patient management, as possible ALS described by the El Escorial criteria is almost always clinically ALS[143,144]. Genetic testing can also be included in patients with a family history of ALS[145] and clinical evidence of disease, although this is not uniformly applied across centres[146].

Cognitive and behavioural deficits

The standard diagnostic and stratification parameters for ALS do not include the cognitive or behavioural status of the patient. Several screening tools have been designed to identify patients with ALS who have cognitive and behavioural changes in the clinic, such as the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), which has been validated in several languages and is widely used, as it has a high degree of sensitivity but lower degrees of specificity[147]. Individuals with abnormal ECAS scores (after adjustment for population-based and educational standards) should be referred for a full neuropsychological evaluation[148]. The detection of cognitive and behavioural changes is important for patients with ALS and their caregivers, as executive impairment is associated with a more-rapid disease trajectory, and behavioural changes are associated with higher caregiver burden[149].

Biomarkers

As ALS is a clinical disease with a heterogeneous phenotypic manifestation and clinical course, diagnostic and prognostic biomarkers are urgently required for stratification. Levels of neurofilament light polypeptide and phosphorylated neurofilament heavy polypeptide in the cerebrospinal fluid (CSF) can differentiate patients with ALS from those with mimics, including cervical myelopathy, multifocal motor neuropathy and inclusion body myositis, with moderate sensitivity and specificity, and levels are correlated with disease progression[150–152]. However, CSF neurofilament levels are not integrated into standard clinical practice. Levels of neurofilament light polypeptide in serum are sensitive and specific for identifying patients with ALS from healthy individuals, but data on whether they can differentiate individuals with ALS from those with mimics are not available.

MRI studies have reported corticospinal tract degeneration, with extensive involvement of the frontal and temporal regions and basal ganglia in patients with ALS, compared with controls. Indeed, selective network vulnerability of structural and functional 'connectomes' might underlie the clinical manifestations of ALS, such as vulnerability of the corticospinal, orbitofrontal, orbitotemporal and frontostriatal circuits[153–155]. The presence of network disruption in ALS is also supported by studies using spectral electroencephalography[129] and findings that patients with different degrees of cognitive impairment have significantly different patterns of frontal lobe metabolic impairment when assessed using ^{18}F–fluorodeoxyglucose PET imaging[156]. However, neither imaging or spectral electroencephalography can provide individualized data that can be used as a reliable biomarker of upper motor neuron dysfunction or of cognitive impairment in patients with ALS.

Differential diagnosis

The differential diagnosis in patients with pure bulbar, pure upper motor neuron or pure lower motor neuron presentations includes ALS variants, treatable ALS mimics and disorders with a more benign prognosis[133,157]. Other forms of motor neuron disease include progressive muscular atrophy (that is, the exclusive degeneration of lower motor neurons) and primary lateral sclerosis (that is, the exclusive degeneration of upper motor neurons). Some patients with progressive muscular atrophy have mutations in genes associated with ALS[158]. Similarly, patients with primary lateral sclerosis might have a family member with ALS, and most autopsies of patients with primary lateral sclerosis show subtle signs of ALS pathology in the lower motor neurons within the brainstem and spinal cord[134,157].

Several conditions have similar presenting features to ALS and should be considered in the differential diagnosis[144], including cervical myelopathy, multifocal motor neuropathy, myasthenia gravis, Lambert–Eaton myasthenic syndrome and inclusion body myositis. Features that should alert the clinician to a possible mimic syndrome include presentation with symmetrical findings, prominent extensor plantar responses (which should raise suspicion of a cervical myelopathy) and the presence of sensory changes. Although sensory symptoms are common in ALS, clinical evidence of sensory loss is atypical and should trigger further investigations. In addition, the presence of substantial weakness in the absence of wasting (which is common in multifocal motor neuropathy and myasthenia gravis) and the disproportionate involvement of the quadriceps (which is common in inclusion body myositis) might indicate the presence of an ALS mimic syndrome[159]. As ALS is a progressive disease, failure of the condition to progress over months should also trigger a reinvestigation[160].

Staging and prognosis

Several different staging systems for ALS have been described[161–164] (FIG. 4), including the King's system, which is based on the number of affected regions of the body, and the Milano–Torino system (MITOS), which is based on a clinical scale. The prognosis of ALS is highly variable, and prognostic algorithms have been generated from population-based and clinical trial-based data sets[165,166]. Negative prognostic indicators include bulbar-onset or respiratory-onset disease, the presence of executive impairment or FTD and weight loss. Several biochemical markers of prognosis have been reported, including serum urate, serum creatinine, serum chloride and increased serum and CSF neurofilament levels[152,167–169]. Worsening respiratory function, assessed by measuring slow vital capacity, forced vital capacity and sniff nasal inspiratory pressure, also correlate with short survival[165,166,170,171].

Clinical genetics and predictive testing

Consensus guidelines recommend the genetic testing of probands with ALS who have a first-degree or second-degree relative with ALS and/or FTD[19,172]. As the genetic risk of ALS depends on ancestral origin, the genetic

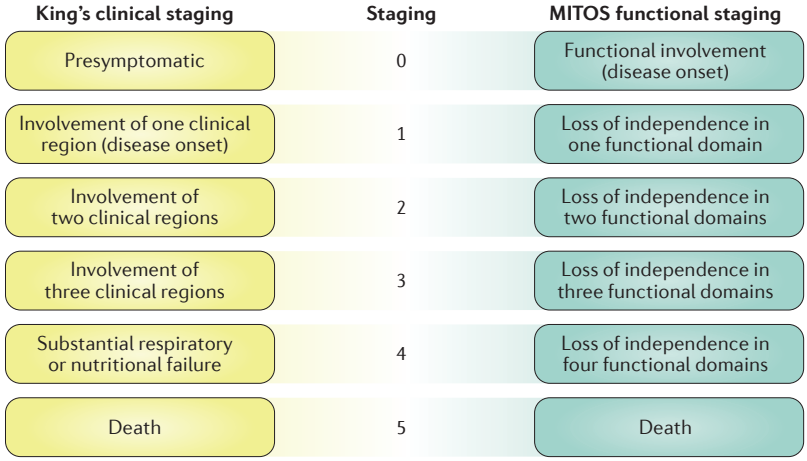

Figure 4 | **Staging systems for ALS.** The King's staging system is based on the number of body regions affected by amyotrophic lateral sclerosis (ALS) and the presence of respiratory or nutritional failure[161]. The Milano–Torino staging (MITOS) system is based on the ALS functional rating scale, a 48-point clinical measurement scale that records changes in four functional domains: bulbar, gross motor, fine motor and respiratory parameters[162]. These staging systems do not incorporate cognitive or behavioural changes. The King's staging system is sensitive to early changes in ALS, but the sensitivity of the MITOS scale is greater in the later stages of disease[163,164].

testing should be contextualized; for example, *C9orf72* variants are rare in Asian populations, whereas mutations in *OPTN* are more common in Asian than in European populations. Although the potential benefits of genetic testing for patients are clear and could improve knowledge of their disease, family planning and their possible inclusion in clinical trials, individuals also have a right not to know their genetic status. Pre-symptomatic testing of family members of patients with ALS remains controversial. Guidelines for genetic testing in research settings have been published[173], but most centres do not advocate routine testing outside of specialist centres[146].

Management

The management of ALS is best achieved by a multidisciplinary approach to care, comprising neurologists, psychologists, nutritionists, pulmonologists, physical therapists, speech therapists and specialized nurses[174,175]. Multidisciplinary care prolongs survival[176–178], reduces the number of hospital admissions, shortens hospital stays[177] and increases quality of life (QOL) of patients with ALS[179]. This finding is likely related to the optimization of pharmacological and non-pharmacological interventions and improved adherence to treatment guidelines.

Disease-modifying therapies

Although >50 drugs with different mechanisms of action have been studied for the treatment of ALS, only two compounds (riluzole and edaravone) have come to market. The negative results of these trials might include clinical and pathogenetic heterogeneity in disease, as well as faults in trial design[180].

Riluzole was the first FDA-approved treatment for ALS and, although the mechanism of action is poorly understood, is speculated to reduce glutamatergic neurotransmission by blocking voltage-gated sodium channels on presynaptic neurons. In the original trial, riluzole increased survival by 3 months, after 18 months of treatment, compared with placebo, but had no significant effect on muscle strength[181]. Riluzole is a relatively safe drug, although the most common adverse effects are an increase in liver enzymes and asthenia (that is, a lack of energy); and some cases of fatal hepatic failure and pancreatitis have been reported. In addition to the traditional tablet form of the drug, an oral suspension has been produced and marketed in some countries for patients who have severe dysphagia[182]. Edaravone, which is thought to act as an antioxidant agent, can slow disease progression in highly selected patients with early onset and rapidly progressing disease[183]; and it has been approved by the FDA but not by the European Medicines Agency. Whether edaravone should be provided to all patients with ALS regardless of clinical presentation is under debate[184].

Symptomatic treatments

The symptoms of ALS can be treated with pharmacological and non-pharmacological interventions. For example, dextromethorphan hydrobromide and quinidine sulfate can improve bulbar function[185] and is available in the United States but not in Europe. However, most of these therapies for the symptoms of ALS have not been tested in randomized controlled trials and are based on the management of other diseases.

Spasticity. Spasticity is present in most patients with ALS, but only a small proportion require treatment. The most commonly used drugs are baclofen and tizanidine (both of which are muscle relaxants), although no randomized controlled trials have been carried out in patients with ALS. When patients have severe, disabling spasticity, baclofen can be administered through an intrathecal pump. Cannabinoids are approved for the treatment of spasticity in patients with multiple sclerosis and are used off-label or as a self-prescribed medication in patients with ALS[186].

Sialorrhoea. Sialorrhoea (that is, hypersalivation), causing drooling and the pooling of saliva within the oral cavity is one of the most disturbing symptoms in patients with ALS and is more commonly observed in patients with bulbar-onset disease and during late stages of disease. Sialorrhoea can be treated with anticholinergic drugs, such as atropine, hyoscine (also known as scopolamine), amitriptyline and glycopyrrolate. Adverse effects associated with the use of anti-cholinergic drugs include blurred vision, dry mouth and constipation, and these drugs are contraindicated in patients with heart conduction disorders and prostatic hypertrophy. In patients in whom pharmacological treatments are ineffective or are not indicated, injections of botulinum toxin A or B into the salivary glands can used to treat sialorrhoea[187,188]. Salivary gland irradiation has also been proposed[189].

Pain. Pain is reported in 15–85% of patients with ALS, depending on the duration of the disease and the setting of the study, and is more commonly nociceptive than neuropathic[190]. Pharmacological treatments include

gabapentin, pregabalin and tricyclic antidepressants (for neuropathic pain) and NSAIDs, opioids and cannabis (for nociceptive pain), but no randomized controlled trials evaluating the treatment of pain in patients with ALS are available. Nociceptive pain can also be treated with intra-joint injections of lidocaine or steroids and with physical therapy, including assistive range-of-motion exercises.

Muscle cramps. Muscle cramps are the main cause of pain in approximately one-quarter of patients with ALS (mainly individuals with spinal-onset disease) and are caused by the instability of motor units[191]. Commonly used treatments for muscle cramps include quinine sulfate, levetiracetam and mexiletine. Indeed, mexiletine significantly reduced the severity of muscle cramps in a dose-dependent manner in a phase II randomized controlled trial in patients with ALS[192]. Of note, the FDA has advised against the use of quinine sulfate for the treatment of muscle cramps because it can cause cardiac arrhythmias, bradycardia and prolongation of the QT interval.

Dysphagia. Dysphagia is reported within 2 years of disease onset by ~60% of patients with spinal-onset ALS and by all patients with bulbar-onset disease[193]. Several strategies can reduce the effects of dysphagia in patients, including dietary changes (such as modification of the consistency of the diet, the use of fluid thickeners, prescriptions for high-protein and high-calorific supplements and manoeuvres to facilitate swallowing) and exercises (such as oral and pharyngeal range-of-motion exercises, head postures and the technique of supraglottic swallow). An option for severe dysphagia is to use enteral nutrition by the insertion of a gastrostomy tube. Established criteria are not available for the initiation of enteral nutrition in patients with ALS, but a weight loss of >5% and unsafe swallowing are generally considered to prompt intervention[174]. Several techniques are available for minimally invasive tube insertion, and open surgery is not recommended[194,195]. Parenteral nutrition administered through a central venous catheter is an alternative to enteral nutrition in patients with ALS who have severe respiratory insufficiency for whom percutaneous endoscopic gastrostomy and radiologically inserted gastrostomy are contraindicated[196,197].

Dysarthria. Dysarthria is the presenting symptom in 30% of patients with ALS and is found in >80% of patients during the course of the disease, up to complete anarthria. Speech therapy can delay the progression of dysarthria, and augmentative and alternative communication techniques (such as customized software) are the treatments of choice and can enhance QOL in the most advanced phases of ALS[198]. Communication techniques based on brain–computer interfaces have been developed, but their use in the clinical setting is still limited, as their effectiveness has not been definitively demonstrated[199]. Moreover, the use of brain–computer interfaces might be hindered by patients' cognitive dysfunction or old age[200].

Deep venous thrombosis. Patients with ALS have leg weakness and reduced mobility, which can increase the risk of symptomatic and asymptomatic deep venous thrombosis. The annual incidence of deep venous thrombosis in patients with ALS ranges from 2.7 to 11.2%[201,202]. In the absence of specific studies on the prevention and treatment of deep venous thrombosis in patients with ALS, general guidelines should be applied, including the use of compression stockings and anticoagulation therapies.

Mood alterations. Depression has been reported in up to 50% of patients with ALS. In general, depression is treated with selective serotonin reuptake inhibitors (SSRIs) or tricyclic antidepressants. Pseudobulbar affect (that is, episodes of uncontrollable crying or laughing) is a distressing symptom that has been reported in up to 50% of patients[203] and can be treated with SSRIs and tricyclic antidepressants, although this use is off-label. Dextromethorphan (a σ-non-opioid intracellular receptor 1 agonist and a non-competitive *N*-methyl-D-aspartate receptor antagonist) and low-dose quinidine reduced the severity of symptoms of pseudobulbar affect by 50% in patients with ALS or multiple sclerosis[204].

Cognitive impairment. Cognitive impairment, in particular FTD, is one of the most disabling symptoms in patients with ALS. No pharmacological therapy is effective for the treatment of FTD, including acetylcholinesterase inhibitors, which are used for Alzheimer disease. However, some symptoms of FTD can be pharmacologically treated; for example, SSRIs might help to control the loss of inhibition, overeating and compulsive behaviour, and antipsychotics can reduce restlessness. The education of caregivers about the symptoms of FTD can be useful to assist in the management of patients at home[205].

Respiratory insufficiency. Most patients with ALS die from respiratory failure. Noninvasive ventilation is the preferred treatment for the symptoms of respiratory failure, and can significantly prolong survival in patients with ALS compared with patients who do not use non-invasive ventilation (316 days versus 229 days) and improves QOL[206,207]. The accepted criteria for commencing noninvasive ventilation are symptoms or signs that are related to respiratory muscle weakness (such as, dyspnoea, orthopnoea or daytime fatigue), a vital capacity of <80% of predicted levels, partial pressure of carbon dioxide of >45 mmHg, or when saturated oxygen is <90% for ≥5% of the time the patient is asleep[175]. One symptom that is related to respiratory muscle weakness in patients with ALS is the inability to cough effectively. This symptom can be controlled using cough-assist devices, such as a mechanical insufflator-exsufflator, or the breath-stacking technique[208].

End-of-life management

The end-of-life phase for patients with ALS can be difficult to define, although recent staging systems, including the King's and MITOS systems, are useful.

The end-of-life period can be particularly challenging and is characterized by substantial mobility, communication and, in some cases, cognitive difficulties. An early discussion of end-of-life issues will ensure that patients can communicate their wishes before the onset of substantial communication and cognitive difficulties, can avoid unwanted interventions or procedures, and can allow time for reflection and the integration of choices within the patient's priorities and life plans. In addition, such discussions can alleviate the patient's fears, especially around fatal choking. The attitudes, culture and personal values of patients, caregivers and health care providers can influence the timing and content of end-of-life discussions, decision-making and the patient's acceptance or refusal of interventions and treatment options. Some patients with ALS might choose life-prolonging measures, but others might contemplate life-limiting procedures; the availability and use of different interventions and technologies, such as assisted death and tracheostomy, varies across centres and between countries. Advance care directives are important at the end of life in patients with ALS, and they provide patients with the option to exercise autonomy regarding preferred end-of-life management strategies. Formal care at the end of life should aim to maximize QOL of the patient and caregiver and, where possible, incorporate appropriate multidisciplinary care, including palliative care options.

Quality of life

Much of the effort of physicians and other health care providers is focused on optimizing the QOL of patients with ALS. The choice of a specific instrument to assess QOL is complex and has been reviewed elsewhere[209]. The perception of QOL of individuals with ALS takes shape at the time of diagnosis, and it can be influenced by the manner in which they are informed. Well-recognized systematic approaches are available to convey the diagnosis in a less distressing manner and to leave the patient feeling hopeful and supported, such as the SPIKES approach[210–212].

Health-related QOL (HRQOL) refers to an individual's perception of their QOL as a function of physical and mental well-being[213] and generally declines in patients with ALS with disease progression[209,214]. By contrast, overall QOL encompasses medical factors and a wide range of non-medical factors, such as family, friends, occupation, financial well-being, spirituality or religion and existential concerns[215]. Healthy individuals usually perceive QOL as being lower in individuals with severe illnesses than those individuals perceive it themselves[216,217]. Patients with ALS often view their overall QOL as good, which persists despite the progression of physical disability[218,219]. This might be explained by a 'response shift' (also called a frameshift or well-being paradox), whereby the individual recalibrates the factors that are deemed meaningful to maintenance of their QOL. Most commonly, this centres around the decreased importance of physical activities and the greater role of interactive and existential factors, such as social relationships and spirituality[220–222]. However,

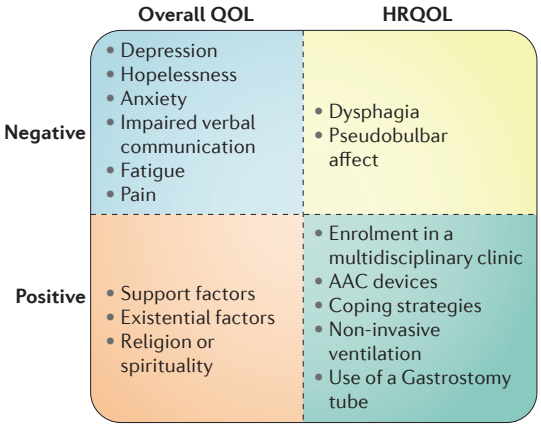

Figure 5 | **Factors affecting QOL in patients with ALS.** Several factors that positively or negatively affect overall quality of life (QOL) and health-related QOL (HRQOL) have been identified in patients with amyotrophic lateral sclerosis (ALS). These factors include the severity of motor symptoms, psychological symptoms and the use of therapeutic interventions. AAC, augmentative-assistive communication.

not all patients maintain a high QOL with advancing illness. Many factors can negatively affect QOL in patients with ALS, which has identified several potential areas for intervention, and other factors can improve QOL[179,206,213,216,223–228] (FIG. 5).

Despite the good QOL of patients with ALS overall, psychological health is, on average, poorer than that of the population as a whole[229]. This finding has substantial implications, as depression, hopelessness and anxiety are all associated with a poor QOL. Psychological interventions to improve QOL have not been well studied in patients with ALS[217], and this warrants further attention.

QOL can affect the wishes for care of patients with ALS at the end of their lives. In a study from the Netherlands, 16.8% of patients with ALS chose physician-assisted death; common reasons for this choice were feelings of hopelessness, a loss of dignity, dependency on others and fatigue[214]. Similarly, the decision for euthanasia in patients with ALS in Washington, United States, was driven by loss of autonomy, participation in enjoyable activities and dignity[215]. These studies do not suggest a poor QOL of these individuals, but they do raise this as a concern. The quality of death in patients with ALS has been studied less comprehensively than QOL. Death was perceived as peaceful by 88–98% of caregivers in Germany, the United Kingdom, the United States and Canada[218,230]. However, caution must be used in interpreting grouped statistics. Incompletely relieved symptoms such as coughing from excess mucus, restlessness, anxiety and muscle cramps resulted in moderate to severe suffering in the last 24 hours of life in 8 of 171 patients[218].

Outlook

The knowledge of ALS and the care of patients with ALS have improved substantially in recent years, and this trend is likely to continue. As of 25 years ago, riluzole had not been enrolled in a clinical trial, non-invasive ventilation

was not in routine use for patients, the pathological basis of ALS as a TDP43 proteinopathy was unknown and genetic causes for ALS had not been identified. In addition, the El Escorial criteria were not developed, no simple ALS functional scale existed, multidisciplinary care was in its infancy, the recognition of cognitive change in patients with ALS was limited and the link with FTD had not been made. It is tempting to consider what will be different in another 25 years, as well as how much of what we regard as self-evident now will be overturned.

Epidemiology

We can expect that the numbers of patients with ALS will increase in the future[219] and that population differences in incidence and phenotype will be recognized. Better multidisciplinary care and an improved understanding of interventions means that a patient diagnosed with ALS can expect to live longer than previously. In addition, the development of new drugs to improve respiratory function or directly affect the disease process is expected to improve survival.

Pathophysiology

A major barrier to effective treatments for ALS is our lack of knowledge of the pathological pathways that lead to the disease and of how they affect the overall integrity of brain networks. Our understanding of ALS is improving, including contextualizing the role of TDP43, the importance of RNA processing in motor neurons, the spread of disease and the molecular cascades that lead to neuronal death. The development of new cellular and animal models of ALS is beginning to lead to improvements in our understanding of the disease, both because the molecular pathways can be studied more easily and because the models can be used to more effectively identify drugs that are worth testing in human trials. These insights are the result of genetic findings, which have led to studies that aim to understand how loss of normal protein function and gain of toxic function cause ALS. As the number of genes implicated in ALS increases and as laboratory models improve, we can expect to design new drugs to intervene in those pathways.

Our understanding of the genetics of ALS has transformed over the past 25 years, with the finding that both familial and sporadic ALS have a genetic basis and with the number of validated genes that have a role in ALS increasing. These findings are largely due to the willingness of the ALS research community to collaborate, which has generated the huge data sets required for credible gene discovery. That the genetic architecture of ALS includes an important role for rare genetic variants has consequences for the effectiveness of gene therapy in this disease. Indeed, as rare variants are more likely to have a large effect on the risk of disease and can be directly manipulated by gene therapy, we expect to see precision medicine spearheaded by targeted gene therapies.

The relationship between ALS and cognitive, cerebellar, autonomic and other non-motor changes is an area of research that is expected to grow. One consequence of this research is that ALS is probably primarily a disease of neural networks that is defined by the involvement of upper and lower motor neurons but that can also affect other cell populations and neuronal networks. We can also expect an increased understanding of the role of inflammation in ALS, both in triggering disease and in influencing the rate of progression.

Diagnosis and prognosis

The use of biomarkers for ALS has been investigated for many years, although our understanding has only recently matured sufficiently to yield useful results. Diagnostic biomarkers would be useful for individuals with an atypical or complicated presentation, prognostic biomarkers would be useful for planning treatment options, and biomarkers of disease progression would be useful for monitoring response to existing therapies or potential new therapies in a clinical trial. New technologies that are based on signal analysis will become available as biomarkers that can image the living human brain[138].

Management

Clinical Trials. The validity of preclinical studies should be evaluated rigorously by evidence-based analyses, and translation of new therapies to humans should be undertaken only if findings from preclinical studies are robust and reproducible. Moreover, as ALS is a human disease, testing safe candidate compounds without previous testing in animal models could be undertaken. In this instance, careful phase I and phase II studies that include detailed pharmacokinetic analysis with extensive dose-finding and analysis of toxicity will be needed. As some previous ALS clinical trials failed due to faulty trial design, a detailed correlative analysis of drug levels in serum and CSF should be undertaken in early-phase trials, and all trials should include a biomarker to confirm that the drug is reaching its target.

The failure of previous clinical trials for ALS could also have resulted from disease heterogeneity. Methods to stratify patients who have a shared pathobiology are urgently required, and in the absence of this, prespecified post hoc analyses should be used to identify potential responder groups. This is exemplified by the successful phase III trial of edaravone[183], as recruitment to this trial was based on a post hoc analysis to identify possible responders, and stringent recruitment criteria were used to identify a clinically homogeneous population that were likely to respond to treatment.

New drugs. An extensive pipeline of new therapeutics for ALS is available, and some of these drugs target known mutations and pathogenetic pathways. Symptomatic therapies, including tirasemtiv, are based on improving respiratory function in patients with ALS and are currently in phase III trials; phase I trials assessing the use of antisense oligonucleotides in *SOD1*-related and *C9orf72*-related ALS are also underway. In the future, treatments are likely to be targeted at specific subgroups of patients and at biomarkers that are personalized to the individual's disease subtype that have been developed from patient cohorts that have been extensively phenotyped and stratified using genomics, transcriptomics, metabolomics and advanced imaging and signal analysis.

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Introduction (O.H.); Epidemiology (G.L.); Mechanisms/pathophysiology (W.R. and P.J.S.); Diagnosis, screening and prevention (O.H. and L.H.v.d.B.); Management (A.C.); Quality of life (Z.S.); Outlook (A.A.-C.); Overview of Primer (E.M.C. and O.H.).

Competing interests statement
O.H. declares grants from the Health Research Board and Science Foundation Ireland and receives funding through the EU Joint Programme in Neurodegenerative Disease Research (JPND), has served on advisory boards for Biogen Idec, Cytokinetics, Orion, Merck and Roche and has consulted for Mitsubishi. She is Editor-in-Chief of the journal ALS and Frontotemporal Degeneration. A.A.-C. has consulted for Biogen Idec, Chronos Therapeutics, Cytokinetics, GlaxoSmithKline, Mitsubishi Tanabe Pharma and Orion Pharma, has received speaking honoraria from Cytokinetics and Lilly, has been the chief or principal investigator of clinical trials for Biogen Idec, Cytokinetics, GlaxoSmithKline and Orion Pharma and receives royalties for the books The Brain (OneWorld Publications) and Genetics of Complex Human Diseases (Cold Spring Harbor Laboratory Press). A.C. has served on scientific advisory boards for Biogen Idec, Cytokinetics, Italfarmaco, Neuraltus and Mitsubishi. G.L. is an Associate Editor of Neuroepidemiology (Karger Publishers). P.J.S. has served on scientific advisory boards for Biogen, Orion Pharma, Sanofi and Treeway and has received research grants from AstraZeneca, Heptares and Reneuron. Z.S. has received consultation fees from Cytokinetics and Neuralstem and research funding from Biogen, Cytokinetics and GlaxoSmithKline. L.H.v.d.B. declares grants from the ALS Foundation Netherlands, grants from The Netherlands Organization for Health Research and Development (Vici scheme), grants from The Netherlands Organization for Health Research and Development (SOPHIA, STRENGTH, ALS-CarE project), funded through the EU JPND, has served on the Scientific Advisory Boards of Biogen, Cytokinetics and Orion and has received honoraria for presentations from Baxalta. E.M.C. and W.R. declare no competing interests.

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CORRECTION

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In the original version of this article, R. Highley was incorrectly stated as R.H. The article has now been corrected.