Amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis is characterised by the progressive loss of motor neurons in the brain and spinal cord. This neurodegenerative syndrome shares pathobiological features with frontotemporal dementia and, indeed, many patients show features of both diseases. Many different genes and pathophysiological processes contribute to the disease, and it will be necessary to understand this heterogeneity to find effective treatments. In this Seminar, we discuss clinical and diagnostic approaches as well as scientific advances in the research fields of genetics, disease modelling, biomarkers, and therapeutic strategies.

Introduction

Amyotrophic lateral sclerosis (ALS) has traditionally been considered a neuromuscular disease, despite the degeneration affecting both upper motor neurons and lower motor neurons. However, compelling clinical, imaging, and neuropathological data have emerged in the past decade, showing more extensive involvement of the CNS than previously recognised. Detailed population-based phenotyping data show that up to 50% of patients with ALS develop cognitive and behavioural impairment, and about 13% of patients have concomitant behavioural-variant frontotemporal dementia.[1,2] Protein aggregates of TAR DNA-binding protein 43 (TDP-43) have been detected in patients with ALS and in patients with frontotemporal dementia.[3]

The discovery of hexanucleotide repeat expansions in *C9orf72* as the major genetic cause of ALS and frontotemporal dementia[4,5] proves that these disorders can be extremes on the phenotypic spectrum of a single disease (figure 1),[6–10] meaning that ALS is a neurodegenerative diseases rather than a neuromuscular disease.

Traditionally, ALS has been classified as either the sporadic or familial form. More than 30 different genes have been linked to the familial form of ALS,[11] which has led to the redefinition of ALS as a clinically and genetically heterogeneous, multidomain neurodegenerative syndrome of motor and extra-motor systems with multiple underlying pathophysiological mechanisms and different clinical subphenotypes.[9] The combination of deep phenotyping, neuroimaging, genomics, and neuropathological assessments will be necessary to fully understand and effectively treat this disease.

Search strategy and selection criteria

We searched PubMed, Google Scholar, and the Cochrane Library for reports published between Jan 1, 1966, and April 20, 2016, using the terms "amyotrophic lateral sclerosis" or "motor neuron disease" or "frontotemporal dementia" in combination with "diagnosis", "epidemiology", "frontotemporal dementia", "imaging", "neurophysiology", "management", "genetics", "biomarkers", "treatment", "C9orf72", and "neuroprotection". We considered additional publications from reference lists and review articles as well as abstracts and reports from relevant meetings. The final reference list was generated on the basis of originality and relevance to the topics covered in this Seminar. Emphasis was placed on reports published within the past 5 years, but we did not exclude commonly referenced and highly regarded older publications.

Epidemiology

The prevalence of ALS in European populations and populations of European descent has been estimated at 2·6–3·0 cases per 100 000 people.[12–15] Lifetime risk is about 1:350 for men and 1:400 for women.[16,17] Few true population-based studies are available from outside of Europe, but the outcomes of the few studies that have been completed indicate differences in the prevalence of ALS between African American, Native Americans, Hispanic, and non-Hispanic of European descent.[18–23] Evidence suggests that the incidence and prevalence of ALS is lower in populations of mixed ancestral origin than in European populations, with differences in age of onset in genetically heterogeneous populations.[15,24–26] In populations of European ancestry, the median age of onset of sporadic ALS is 65 years, whereas the mean age of onset in genetically heterogeneous populations is about 10 years earlier.[13,14,26–28] Although analysis of population-based registers has not indicated substantial changes in the adjusted age-specific incidence with time, the growing recognition of a continuum between ALS and frontotemporal dementia seems to have subtly shifted the types of patients who are included in registers, which could partly explain the observed increase in the incidence of ALS, particularly in people at late stages of life.[19,29,30] In most population-based studies, ALS is found to be more common in men than in women, affecting 1·2–1·5 men for every woman.[12–15] By contrast with Alzheimer's disease, the risk of developing ALS peaks at 50–75 years of age and decreases thereafter. Survival is highly variable, but respiratory failure usually leads to death about 3–4 years after onset.[12–15]

Clinical presentations and diagnosis

ALS is characterised by progressive motor deficits that develop within weeks or months. Any voluntary muscle can be affected, resulting in heterogeneous

presentations ranging from dysarthria to a foot drop (table 1).[9] However, motor neurons in the oculomotor nuclei and in Onuf's nucleus appear to be resistant, and eye movement and sphincter control therefore remain unaffected. Both upper motor neuron and lower motor neuron signs are present on neurological examination (figure 1). Disease onset is usually focal, but the disease eventually spreads to other body regions. The progression and spread of the disease appears to be both local (within the same region; eg, from hand to upper arm) and between neuroanatomically linked regions (contra-lateral or rostral-caudal).[31]

The heterogeneous clinical presentation and varying speed of progression make diagnosis of ALS challenging. No diagnostic test exists to definitively demonstrate ALS, and the various differential diagnoses and investigations must therefore be tailored to each individual patient. The El Escorial or Awaji diagnostic criteria (primarily used for research; figure 2)[32,33] are used for patients who have a history of progressive weakness that has spread within a region or to other regions (bulbar, cervical, thoracic, or lumbar), with evidence of lower motor neuron (clinical or electrophysiological) and upper motor neuron (clinical) involvement, and that no other disease processes explain the presentation.[32–35]

The disease is often classified by site or pattern of onset or by degree of upper motor neuron or lower motor neuron involvement, which has prognostic value (table 2) and helps structure the differential diagnosis and diagnostic assessment (figure 3).[36]

ALS variants

Diagnosis of ALS is relatively straightforward when upper motor neuron and lower motor neuron signs are clearly present in multiple regions and when other diagnoses have been excluded by imaging and neurophysiological examination. However, at onset, upper motor neuron signs can predominate and lower motor neuron involvement might only become evident at a later stage, or vice versa. In these cases, the differential diagnosis is more extensive and includes ALS variants, treatable ALS mimics, and disorders with a more benign prognosis.[37] Recognising these mimics and variants is therefore important (figure 4; appendix).

The El Escorial criteria includes restricted forms of ALS: progressive spinal muscular atrophy (exclusively lower motor neuron degeneration) and primary lateral sclerosis (exclusively upper motor neuron degeneration).[35] Whether these are indeed separate diseases or two forms of ALS is a longstanding topic of debate, particularly for progressive spinal muscular atrophy. Autopsies from patients with progressive spinal muscular atrophy have shown corticospinal tract involvement.[38] Some patients with progressive spinal muscular atrophy carry mutations in genes associated with ALS[39] and might have cognitive involvement,[40] and patients in ALS pedigrees might have pure lower motor neuron phenotypes.[9]

Similarly, upper motor neuron degeneration in primary lateral sclerosis leads to progressive and disabling spasticity but is rarely associated with respiratory failure. The prognosis of primary lateral sclerosis is therefore more benign than ALS (from more than 10 years to normal lifespan) and important to diagnose.[41] The main challenge is to distinguish between primary lateral sclerosis and upper motor neuron-predominant ALS, which usually progresses to

See **Online** for appendix

	Distribution	Clinical characteristics
Classic ALS (70%)*		
Bulbar (33%)	Bulbar with involvement of other regions	Dysarthria is the presenting feature in all patients with bulbar-onset ALS, and dysphagia usually develops later (although can develop simultaneously) in the disease; bulbar upper motor neuron signs include exaggerated jaw jerk, pseudobulbar affect, and spasticity; bulbar lower motor neuron signs include tongue wasting (never asymmetrical) and fasciculations; patients with bulbar onset generally present with both upper and lower motor neuron signs
Spinal (66%)	Flail arm Flail leg Hemiplegic Pseudopolyneuritic	Lower motor neuron involvement proximally in the arms, often with mild upper motor neuron signs in the legs Lower motor neuron involvement restricted to the legs, usually asymmetrical Progressive, unilateral upper motor neuron involvement with facial sparing, sometimes with discrete lower motor neuron involvement Predominantly distal lower motor neuron signs in the limbs with limited upper motor neuron involvement
ALS–FTD (5–15%)†		
Bulbar or spinal	Distribution as in classical ALS	Classic ALS with a spinal or bulbar onset, but also signs of cognitive or behavioural changes, or both, fulfilling the diagnostic criteria for FTD (5–15% of ALS patients); patients most commonly have behavioural variant FTD with apathy and loss of sympathy as the commonly affected behavioural domains; semantic dementia is also seen; the non-fluent and logopenic variants are very rare or not encountered; take a careful family history and explicitly ask for dementia, Parkinson's disease, psychiatric disease, suicide, and addiction; associated with repeat expansions in *C9orf72*
Isolated bulbar involvement (5%)		
Pseudobulbar palsy, isolated bulbar palsy		
Bulbar	Bulbar only	Some patients present with bulbar signs that remain restricted to the bulbar region for an extended period of time (years) without spreading to other regions (as would be seen in bulbar-onset ALS); patients are predominantly women, have a spastic dysarthria, and commonly have emotional lability
Restricted phenotypes of ALS (10%)		
Progressive spinal muscular atrophy (only lower motor neuron involvement)		
Spinal	Spreading from a focal onset or patchy	Generalised lower motor neuron involvement; onset can be focal or patchy, but there is clear progression to other regions with time, eventually leading to respiratory failure; average survival is longer than for classical ALS; patients should be followed regularly as upper motor neuron involvement can become apparent during the disease course

(Table 1 continues on next page)

	Distribution	Clinical characteristics
(Continued from previous page)		
Primary lateral sclerosis (only upper motor involvement)		
Bulbar or lower limbs	Spread from bulbar to limbs, from legs to arms and bulbar region; can be one-sided (Mill's syndrome)	Exclusive upper motor neuron signs for more than 4 years; in upper motor neuron-predominant ALS, lower motor neuron signs can become evident with time; when upper motor neuro signs are symmetrical and limited to the legs (sporadic), hereditary spastic paraplegia is an important diagnostic consideration; survival ranges from more than 10 years to normal life expectancy
Rare phenotypes (3%)		
Cachexia	Develops into classic ALS	Unexplained weight loss may precede upper motor neuron or lower motor neuron signs, or both
Respiratory onset, diaphragm and neck flexors	Diaphragm and neck flexors	Usually referred by cardiologist or pulmonologist; initial consult is often in the intensive care unit; weakness of diaphragm and neck flexors; associated with poor prognosis

ALS=amyotrophic lateral sclerosis. FTD=frontotemporal dementia. *Signs of upper motor neuron or lower motor neuron, or both, in multiple regions at presentation. †ALS-FTD refers to patients who fulfil the diagnostic criteria for both ALS and FTD.

***Table 1*: The distribution and clinical characteristics of the most common presentations of amyotrophic lateral sclerosis, by designation and site of onset**

a more generalised form of ALS within 4 years. Pure forms of hereditary spastic paraplegia are an important diagnostic alternative to primary lateral sclerosis. Hereditary spastic paraplegia is usually familial, with early and symmetrical onset and limited or no involvement of the arms. Disease progression is usually slower than primary lateral sclerosis, and bulbar involvement is rare. Genetic testing for genes associated with hereditary spastic paraplegia should be performed, and in some cases the correct diagnosis only becomes evident by follow-up.[41–43]

Cognitive and behavioural changes

Cognitive and behavioural changes are an intrinsic component of some forms of ALS. The approach is to first make a definitive diagnosis of ALS and to subsequently screen for cognitive and behavioural changes. 5–15% of patients with ALS also have frontotemporal dementia, and up to 50% of patients with ALS have cognitive or behavioural changes within the spectrum of frontotemporal dementia.[1,2,9,44] Similarly, 12·5% of patients with behavioural-variant frontotemporal dementia develop ALS, and mild motor neuron

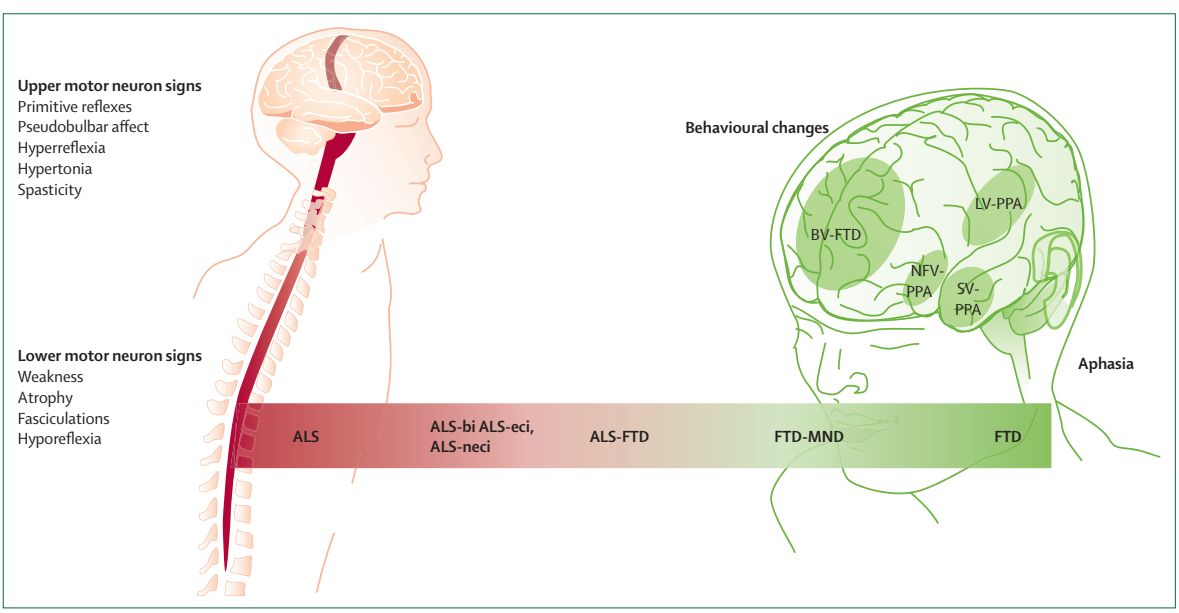

***Figure 1*: Amyotrophic lateral sclerosis and frontotemporal dementia—extremes on the phenotypic spectrum of a single disease**
Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) might be phenotypic extremes on a spectrum disorder (the so-called motor neuron disease–FTD continuum). About half of all patients with ALS only have motor involvement (classical ALS). Those patients with intact cognitive function at diagnosis appear to maintain cognitive function during the disease course. However, up to half of patients with ALS show some degree of cognitive impairment or behavioural changes, but without fulfilling the diagnostic criteria for FTD. The disease is categorised as ALS-eci if there is evidence of executive dysfunction, ALS-neci if there is no executive dysfunction but impairment in other cognitive domains (eg, memory), or ALS-bi if behavioural changes are present. About 5–10% of patients with ALS also have FTD (most often the behavioural variant). Patients with motor neuron disease–FTD have a primary diagnosis of FTD; motor neuron involvement develops as the disease progresses but not to full ALS. FTD can be divided into two subtypes; behavioural variant and the primary progressive aphasias (PPAs), which are characterised by language deficits. The PPAs can be further subdivided into three forms: the non-fluent variant, semantic variant, and logopenic variant. With time, patients who present with the behavioural variant of FTD develop language deficits and vice versa. The PPAs in certain FTD subtypes have very specific neuroanatomical correlates within the language network (left posterior frontal and insular regions for the non-fluent variant; anterior temporal region for the semantic variant; and left temporo-parietal regions for the logopenic variant). ALS appears to be more closely related to the behavioural variant of FTD than the PPAs. ALS=amyotrophic lateral sclerosis. FTD=frontotemporal dementia. ALS-eci=ALS with evidence of executive dysfunction. ALS-neci=ALS with no executive dysfunction but impairment in other cognitive domains. ALS-bi=ALS with behavioural changes. BV-FTD=behavioural variant of FTD. PPA=primary progressive aphasias. NFV=non-fluent variant. SV=semantic variant. LV=logopenic variant.

Diagnostic criteria for ALS (revised El Escorial criteria)	
Definite ALS	UMN and LMN signs in three regions
Probable ALS	UMN and LMN signs in two regions, with some UMN rostral to LMN signs
Probable, laboratory-supported ALS	UMN signs in at least one region with EMG evidence of LMN loss in two regions
Possible ALS	UMN and LMN signs in one region, or UMN signs in two regions, or UMN and LMN signs in two regions but no UMN rostral to LMN signs

Criteria for frontotemporal cognitive and behavioural syndromes in ALS	
ALS-FTD	Meets criteria for bv-FTD or non-fluent, semantic, or logopenic variant primary progressive aphasia
ALS-bi	Meets two of six criteria for bv-FTD
ALS-eci	Impairment on two tests for executive function
ALS-neci	Impairment on two non-executive domains (memory or visuospatial functions)
FTD-MND	Primary diagnosis of FTD with neuropathological evidence of motor neuron degeneration

Criteria for frontotemporal dementia				
	Primary progressive aphasia			Behavioural variant of FTD
	Non-fluent variant	Semantic variant	Logopenic variant	
1. Clinical diagnosis	One of the following criteria must be present	Both criteria must be present	Both criteria must be present	At least three of the following criteria must be present
	1. Agrammatism in language production 2. Effortful, halting speech with inconsistent speech sound errors and distortions (apraxia of speech)	1. Impaired confrontation naming 2. Impaired single-word comprehension	1. Impaired single-word retrieval in spontaneous speech and naming 2. Impaired repetition of sentences and phrases	1. Early behavioural disinhibition 2. Early apathy or inertia 3. Early loss of sympathy or empathy 4. Early perseverative, stereotyped, or compulsive or ritualistic behaviour 5. Hyperorality and dietary changes 6. Executive deficits
	At least two of the following criteria must must be present	At least three of the following criteria must be present	At least three of the following criteria must be present	
	1. Impaired comprehension of syntactically complex sentences 2. Spared single-word comprehension 3. Spared object knowledge	1. Impaired object knowledge 2. Surface dyslexia or dysgraphia 3. Spared repetition 4. Spared speech production (grammar and motor speech)	1. Speech (phonologic) errors in spontaneous speech and naming 2. Spared single-word comprehension and object knowledge 3. Spared motor speech 4. Absence of frank agrammatism	
2. Imaging supported	Fulfils clinical criteria, clear progression, and atrophy on MRI or hypoperfusion or hypometabolism on PET or SPECT			
	Left posterior fronto-insular area	Anterior temporal lobe	Left posterior perisylvian or parietal lobe	Frontal or anterior temporal lobe, or both
3. Definite pathology	Fulfils clinical criteria and one of the following pathological criteria			
	Histopathological evidence of a specific neurodegenerative pathology (eg, FTLD-tau, FTLD-TDP)			
	Presence of a known pathogenic mutation			

***Figure 2*: Diagnostic criteria for the amyotrophic lateral sclerosis and frontotemporal dementia**
ALS=amyotrophic lateral sclerosis. FTD=frontotemporal dementia. FTD-MND=frontotemporal dementia ALS-eci=ALS with evidence of executive dysfunction. ALS-neci=ALS with is no executive dysfunction but impairment in other cognitive domains. ALS-bi=ALS with behavioural changes. FTLD-tau=frontotemporal lobar degeneration with tau protein inclusions. FTLD-TDP=frontotemporal lobar degeneration with TDP-43 protein inclusions.

involvement is seen in about 40% of patients with frontotemporal dementia.[45,46] The diagnostic criteria for frontotemporal dementia apply to patients with ALS as they would to any other patient (figure 2).[47,48] Disease presentations with cognitive or behavioural changes that do not fulfil formal diagnostic criteria can be grouped into one of three categories: ALS with behavioural impairment; ALS with executive dysfunction; and ALS non-executive dysfunction (figure 2).[49]

Many conventional neuropsychological tests rely on patients being able to speak and write so they might not be suitable for use in ALS diagnosis. Several screening tools specifically designed for ALS are now available and include the ALS-Brief Cognitive Assessment (ALS-BCA),[50] the ALS-Cognitive Behavioral Screen (ALS-CBS),[51] the ALS-FTD-Q,[52] and the Edinburgh Cognitive and Behavioral ALS Screen (ECAS).[53] Patients with abnormal scores on these screening tests should be referred for full neuropsychological assessment.

Apathy and loss of sympathy are the most common behavioural symptoms, affecting about 10% of all patients with ALS.[53] Fluency, language, social cognition, and executive function are the cognitive domains that are most often affected. Memory impairment is also

	Associated with long survival	Associated with short survival
Clinical features	Flail arm variant; lower motor neuron-predominant disease; upper motor neuron-predominant disease; long time to diagnosis; young age at diagnosis	Bulbar-onset ALS; respiratory onset; executive dysfunction and comorbid FTD; poor nutritional status; neck flexor weakness; old age at diagnosis
Genetic factors	*SOD1* mutations: Glu22Gly, Gly38Arg, Asp91Ala, Gly94Cys, and Ile114Thr; reduced *EPHA4* expression	Ala5Val mutation in *SOD1*; repeat expansions in *C9orf72* or *ATXN2*; mutations in *FUS* (also associated with early onset); homozygosity for the C allele of rs12608932 in *UNC13α*
Environmental and life style factors	None	Low socioeconomic status; smoking
Treatments	Riluzole treatment; non-invasive ventilation; enteral feeding; moderate exercise; multidisciplinary clinic care	Carbamazepine; minocycline; diaphragm pacing

ALS=amyotrophic lateral sclerosis. FTD=frontotemporal dementia.

***Table 2*: Prognostic factors in amyotrophic lateral sclerosis**

	Distribution and key findings		Most likely diagnosis or differential diagnosis	Next steps or confirmation of diagnosis
Isolated LMN involvement	Cramps and fasciculations, no weakness	Normal EMG	Cramp-fasciculation syndrome	Symptomatic treatment
	Distal symmetrical	EMG	Length dependent, axonal: CMT type 2, dHMN	Genetic tests (CMT genes, *HSPB1* and *HSPB8*)
	Proximal symmetrical	EMG	Increment: LEMS	Anti-VGCC Ab, chest CT (lung carcinoma)
			Myopathic: endocrinopathy, myopathies (IBM, SLONM, Pompe disease, MD)	Laboratory tests , muscle biopsy, genetic tests
			Demyelinating: CIDP	Lumbar puncture: no pleiocytosis → start treatment (immunoglobulins, steroids)
			Neurogenic: Kennedy's disease, SMA type 4, PMA, ALS	Genetic tests (*AR, SMN1, VAPB* genes), follow-up
	Asymmetrical	History	M-protein: lymfoproliferative disorder	Refer to haematologist
		Examination	Painful at onset: diabetic amyotrophy, brachial neuritis	Glucose, HbA_{1c}, EMG, follow-up
		Laboratory test	Medical history: post-polio syndrome, post-radiation	Symptomatic treatment
			Quadriceps and finger flexors: IBM	Muscle biopsy (inflammation, rimmed vacuoles), anti-cN1A Ab
		EMG	Neurogenic: Hirayama's disease, Segmental SMA, PMA, ALS	C-spine MRI, EMG, cognitive tests, follow-up
			Demyelinating: MMN, Lewis-Sumner syndrome	Lumbar puncture: no pleiocytosis → start treatment (immunoglobulins)
Isolated bulbar involvement	UMN signs	Normal MRI	PBP, PLS, ALS	EMG, cognitive tests, follow-up
		Abnormal MRI	Bilateral corticobulbar tract damage (ischaemia), other	In accordance with MRI findings
	Fluctuating or ocular involvement		Myasthenia gravis	AchR Ab, MuSK Ab, single fibre EMG, chest CT (thymoma)
	Cranial nerve involvement		Villaret syndrome, syringobulbia, basal meningitis, FOSMN	MRI, lumbar punction, MRI, EMG (blink reflex)
	Perioral fasciculations		Kennedy's disease	Genetic test (*AR*)
Isolated UMN involvement	Standard neuro-axis MRI	Normal MRI	Bulbar involvement: PBP, PLS, ALS	EMG, cognitive testing, follow-up
			No bulbar involvement: PLS, ALS, HSP	Genetic testing (HSP genes), follow-up
		Abnormal MRI	ppMS, leucodystrophy, mass lesion: eg, falx meningeoma	In accordance with MRI findings
	Metabolic screening		Krabbe's disease, CTX, AMN, other	
	Serology		Infectious: HTLV-1 , Lyme's disease, HIV, syphilis	Antibiotics, anti-retroviral treatment, consider steroids for HTLV-1
	LMN involvement on EMG		ALS	EMG, cognitive tests, follow-up
UMN and LMN involvement	No UMN signs rostral to LMN signs		Myelopathy	C-spine MRI, vitamin B12
	UMN and LMN signs in three regions		ALS	Cognitive testing, follow-up
	Serology		Infectious : Lyme's disease, HIV, syphilitic amyotrophy	Antibiotics, anti-retroviral treatment
	Concomitant peripheral neuropathy		AMN, vitamin B12 deficiency, unrelated neuropathy	Laboratory investigations, long fatty acids, genetit test (*ABCD1*)
	Low TSH		Hyperthyroidism	Refer to endocrinologist
	Consider two unrelated conditions			

***Figure 3*: Diagnostic assessment of a patient suspected of having amyotrophic lateral sclerosis**
The first step is to categorise the phenotype by lower motor neuron involvement, upper motor neuron involvement, both upper and lower motor neuron involvement, or bulbar involvement. Subsequently, the distribution of signs (distal *vs* proximal, symmetrical *vs* asymmetrical), certain clinical features (eg, gynaecomastia), and results from ancillary investigations will guide the diagnosis. Additional diagnostic tests should be done in accordance with the clinical presentation, but generally, when there is a suspicion of amyotrophic lateral sclerosis, it is recommended to do laboratory investigations (including measurements of creatine kinase activity, thyroid function, vitamins, M-protein, electrolytes, full blood count), serology tests for HIV, Lyme disease, and syphilis, MRI imaging as appropriate, and needle EMG. Genetic testing will probably become more common in the diagnostic assessment of amyotrophic lateral sclerosis. LMN=lower motor neuron. UMN=upper motor neuron. EMG=electromyography. CMT=Charcot-Marie Tooth disease. dHMN=distal hereditary motor neuronopathy. LEMS=Lambert-Eaton myasthenic syndrome. VGCC= voltage-gated calcium channels. Ab=antibody. IBM=inclusion body myositis. SLONM=slow late onset nemalin myopathy. MD=myotonic dystrophy. CIDP=chronic inflammatory demyelinating polyneuropathy. SMA=spinal muscular atrophy. PMA=progressive spinal muscular atrophy. ALS=amyotrophic lateral sclerosis. HbA_{1c}=glycated haemoglobin. cN1A=cytosolic 5′-nucleotidase 1A MMN=multifocal motor neuropathy. PBP=pseudobulbar palsy. PLS=primary lateral sclerosis. AChR= acetylcholine receptor. MuSK= muscle specific kinase. FOSMN=facial onset sensory motor neuropathy. HSP=hereditary spastic paraplegia. ppMS=primary progressive multiple sclerosis. CTX=cerebrotendineous xanthomatosis. AMN=adrenomyeloneuropathy. HTLV-1=human T-cell lymphotropic virus type 1. *ABCD1*=ATP binding cassette subfamily D member 1. TSH=thyroid stimulating hormone.

occasionally detected but rarely exists in isolation.[54] Very few longitudinal studies on cognition in ALS have been performed. Patients without deficits at diagnosis remain unaffected, and cognitive decline in patients with non-executive dysfunction tends to be slow. Executive dysfunction, however, is associated with rapid disease progression.[55]

Recognising cognitive and behavioural impairment is important because it is associated with genetic mutations (eg, *C9orf72, TBK1*), aggressive disease, non-compliance with treatment recommendations, and increased caregiver burden.[50,51] Moreover, as impairment in capacity affects medico-legal decision making, power of attorney should be discussed early for patients with cognitive changes, behavioural changes, or both.[56]

Pathophysiology

The mechanisms underlying neurodegeneration in ALS are still not fully understood. Many cellular and molecular processes have been implicated, including mitochondrial dysfunction, axonal transport, toxic protein aggregation, impaired protein degradation (involving the proteasome

or autophagy, or both), prion-like spreading, excitotoxicity, decreased neurotrophic support from non-neuronal cells, oxidative stress, hypermetabolism, inflammation, RNA metabolism defects, and RNA toxicity. The evidence for these mechanisms has been described extensively.[8,44] Defects in some of these pathways could be secondary phenomena, and genetics would be the logical initial approach to identifying the primary pathophysiological processes underlying ALS.

In 5–15% of patients with ALS, the ALS or frontotemporal dementia runs in the family.[9,57,58] In these cases a single genetic defect is thought to cause disease. Functionally, the 30 genes associated with familial ALS[11] can be grouped into three main pathophysiological processes—RNA biology, protein turnover, and axonal transport, suggesting that deficits in these pathways are causal.[8] However, most patients do not have a family history of ALS, in which case the disease is thought to have sporadically resulted from both environmental and genetic risk factors.[17] Multiple genetic risk factors for sporadic ALS have been identified. The search for environmental risk factors has, however, been less fruitful. Many case-control studies of exposure risks have been confounded by methodological errors and low power. High incidences of ALS have been recorded in Guam and the Kii Peninsula (Japan), and associations with cyanobacterial neurotoxins (β-Methylamino-L-alanine) have been proposed but never confirmed.[59–61]

Clustering of ALS has been reported among Italian soccer players and American football players,[62,63] and investigators have done detailed population-based, case-control studies to seek an association between physical exercise and ALS, but with conflicting results.[64,65] Risk could be conferred by the factors that determine an athletic disposition, rather than the actual exercise. Other proposed environmental risk factors include smoking, exposure to pesticides and organic toxins, and electromagnetic radiation.[17] With the exception of smoking,[66] definitive evidence of risk remains to be established and will rely on large, unbiased, population-based case-control studies for confirmation.

The high degree of variability in phenotype and family history and the many genes, molecular pathways, and environmental risk factors that have been associated with ALS suggest that different mechanisms underlie neurodegeneration in different patients. Some evidence suggests that multiple pathways are in fact necessary to develop ALS.[67] Analysis of population-based registers revealed a log-linear relationship between incidence and age of onset, which, similar to cancer, is consistent with a multistep model of disease. In this model, six steps are estimated to be sufficient to cause disease, and each step is a change to a distinct pathophysiological process, the last of which triggers the disease. These findings emphasise the need to study genetic, environmental, and lifestyle risk factors.[67] Although the multistep model is still only a hypothesis, it is consistent with many features of ALS, including the phenotypic variability, the late onset, non-penetrance, genetic pleiotropy, and the process by which the disease cascades through the motor system rapidly after onset.

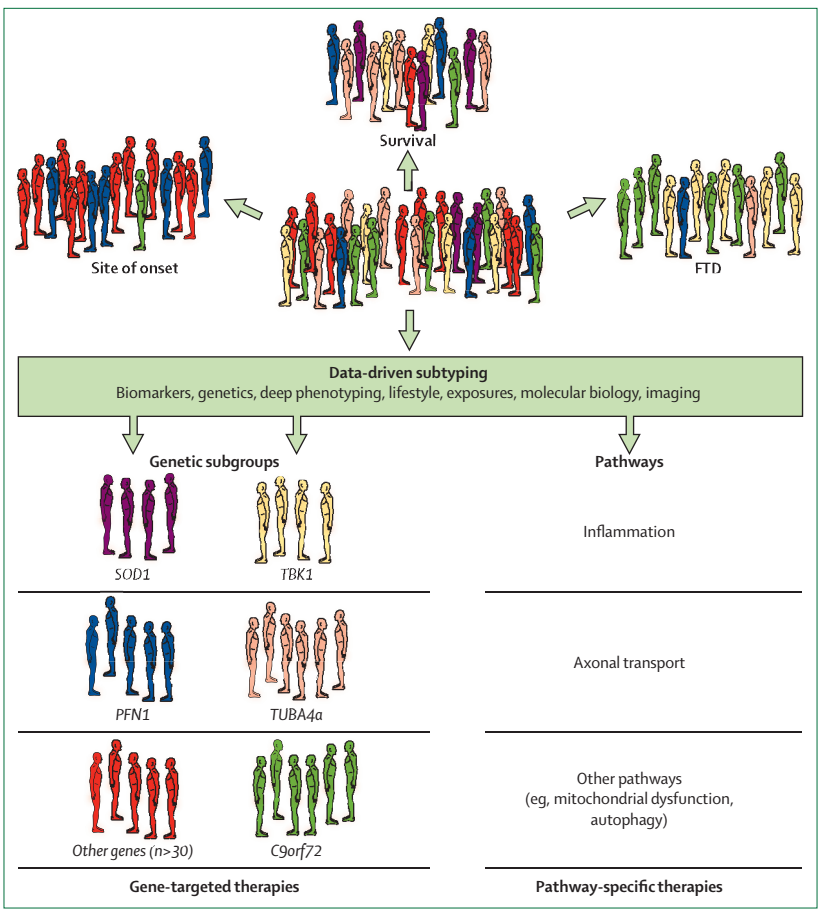

Figure 4: **Moving towards precision medicine in amyotrophic lateral sclerosis**
Patients with amyotrophic lateral sclerosis (ALS) are included in drug trials without considering genetics or the possibility of varying underlying pathophysiology between patients. Post-hoc analyses are often done on clinical characteristics, such as site of onset or the presence of comorbid frontotemporal dementia. Considering the extensive heterogeneity of ALS, it seems highly plausible that different subtypes of ALS will require different treatments. Unravelling this heterogeneity through the combined approaches of deep-phenotyping, imaging, genetics to analyse the effects of life style factors, exposures, and molecular biological characteristics will identify homogeneous groups of patients and facilitate more targeted treatment approaches (either gene or pathway specific).

Although multiple mechanisms appear to be at play, abnormal aggregation of TDP-43 is a recurring pathological feature in nearly all patients with ALS (except for patients carrying mutations in *SOD1* and *FUS*), suggesting that altered TDP-43 function is an important disease-causing factor.[3,68] TDP-43 normally localises to the nucleus where it functions in transcription, but misfolded TDP-43 aggregates in the cytosol, leading to a nuclear loss-of-function that might cause transcription deficits. TDP-43 aggregates might also acquire toxic properties through increased hydrophobicity and sequestration of essential cellular components, generation of oxidative species, and proteasome inhibition.

Mounting evidence suggests that these aggregates might spread through a self-perpetuating or prion-like

mechanism. The initial misfolding of TDP-43, SOD1, and FUS proteins is perhaps aggravated under certain conditions (eg, cell stress). These newly misfolded proteins (seeds) are in turn capable of misfolding their native counterparts, thereby initiating a cascade.[69–71] SOD1 has been shown to form seeds that can spread to neighbouring cells and through neuroanatomical pathways, which possibly reflects the clinical spread of disease.[71] Cell-to-cell transmission of dipeptide repeat proteins via exosomes has also been linked to *C9orf72* repeat expansions.[72]

Several viral infections (eg, HIV, polio virus) can cause motor neuron dysfunction, but there is no evidence that ALS is due to viral infections. However, a substantial part of the human genome (about 8%) is remnant DNA from retroviral infections that occurred in distant ancestors and was incorporated into the germline. Most of the viral genes have become defective through the accumulation of nonsense mutations. Yet some reverse transcriptase activity has been detected in serum of patients with ALS, possibly due to reactivated endogenous retrovirus genes rather than a new infection.[73–75] Expression of the human endogenous retrovirus K (HERV-K) gene has been detected in cortical and spinal neurons from a subpopulation of patients with ALS but not in healthy controls. The HERV-K genome encodes three genes, one of which encodes an envelope protein (*env*) that is selectively toxic to motor neurons in mouse models. HERV-K genes are regulated by TDP-43, raising the possibility that changes in TDP-43 concentrations could reactivate inherited retroviral genes, causing HERV-K *env* expression and subsequent neurodegeneration.[76] Two clinical trials of anti-retroviral therapy to suppress HERV-K in patients with ALS are ongoing (NCT02437110 and NCT02868580).

Both the prion and the endogenous retroviral reactivation hypotheses pose interesting explanations for the manner in which ALS spreads after onset and could be the final step in the multistep model.

For the **Project MinE** see https://www.projectmine.com

Genetics

In about 60–80% of patients with familial ALS, a mutation of large effect (presumably pathogenic) can be identified, of which *C9orf72* (40%), *SOD1* (20%), *FUS* (1–5%), and *TARBDP* (1–5%) are the most common.[11]

The genetics of sporadic ALS is less well understood. Findings from twin studies show that the genetic contribution to sporadic ALS is 61% (95% CI 38–78%).[77,78] In one genome-wide association study,[79] the genetic architecture of the disease was analysed by partitioning the explained heritability by allele frequency; the results showed that the remaining genetic risk factors are disproportionately likely to be rare variants (0·1–5%), with intermediate to large effects. These findings imply that ALS is an oligogenic disease and therefore distinct from many common disorders and neuropsychiatric disorders such as schizophrenia, which are highly polygenic (because of the additive effect of many common genetic polymorphisms with small effects).[80] An oligogenic model is consistent with the incomplete penetrance in many ALS pedigrees, the reduced rate of ALS in genetically heterogeneous populations, and the cosegregation of multiple ALS-associated genes with disease in some kindreds.[81–83] Heritability can also be obscured in small pedigrees (death resulting from other causes before the onset of ALS, loss of contact, etc), causing familial cases to appear sporadic.[84] About 10% of patients with sporadic ALS have mutations in genes that are known to be associated with familial ALS, and first-degree relatives of patients with sporadic ALS are at an eight-fold higher risk of developing disease.[85] Rigid dichotomising of ALS into familial and sporadic disease is now considered an over-simplification, as all evidence points towards similarities in genetic architectures between familial and sporadic disease. Moreover, many genes associated with ALS are pleiotropic. The most established example of pleiotropy is *C9orf72,* which is clearly linked to ALS and frontotemporal dementia but is also linked to Parkinsonism, Huntington phenocopies, Alzheimer's disease, corticobasal degeneration, schizophrenia, and bipolar disorder.[10] Other examples of pleiotropy are repeat expansions in *ATXN2* (associated with spinocerebellar ataxia type 2, ALS, and parkinsonism)[86,87] and *ANG* (associated with ALS and parkinsonism).[88,89] Mutations in *hnRNPA1, hnRNPA2b1, SQSTM1,* and *VCP* have been detected in family pedigrees with heterogeneous phentoypes (ALS, frontotemporal dementia, inclusion body myositis, and Paget's disease of the bone [also known as multisystem proteinopathy]).[90–92] Other genes, including *Matr3*, *CHCHD10*, and *SQSTM1* have also been implicated in myopathies.[93–95]

Considering the genetic architecture of ALS, whole-genome sequencing of large numbers of patients and controls will probably be necessary to fully unravel the underlying genetics. In 2012, Project MinE launched an international whole-genome sequencing project to sequence the complete genomes of 15 000 ALS cases and 7500 controls; this project is estimated to be completed by early 2018. Genetic testing in clinical practice is discussed in the panel.

From genes to biology

For a long time *SOD1* was the only gene to be associated with ALS, and transgenic *SOD1* mice were the only available ALS animal model of the disease.[109] Although this mouse model shows several ALS phenotypes, it is probably not representative for most forms of ALS because pathological TDP-43 accumulation is not present (table 3). This might explain why translation of therapeutic approaches from mouse model to patients has been difficult.[110]

Multiple ALS models now exist for different genes (eg, *TARDBP*, *FUS*, *C9orf72*, *VAPB*, *VCP*) in different species (*Caenorhabditis elegans*, *Drosophila melanogaster*,

Danio rerio, *Mus musculus*, and *Rattus norvegicus*).[109,111–115] Similar to the transgenic *SOD1* mouse, these model organisms often do not display all features of patients with ALS who carry corresponding mutations, but they have nevertheless been extremely valuable for the investigation of gene mutations and their effects at the molecular, cellular, and systems levels. With ongoing gene discovery and the development of powerful genome editing tools such as CRISPR/Cas9, many more ALS models are expected in the coming years. Stem-cell-based model systems have also become important in ALS research. The ability to convert human somatic cells (eg, skin fibroblasts) into induced pluripotent stem cells has revolutionised research of human disease.[116] This technology has already been used to generate patient-derived motor neurons and to detect cellular defects such as impaired neurotransmission, cell death, and altered neuronal morphology.[117]

Because the genetic and epigenetic makeup of patients are preserved in human motor neurons derived from induced pluripotent stem cells, these cultures are viewed as promising models for future screening of therapeutic compounds.[117] For example, three (non-mutually exclusive) mechanisms underpinning *C9orf72*-related pathophysiology have been described using these models and techniques. One mechanism involves haploinsufficiency, which is supported by evidence of reduced *C9orf72* mRNA and protein in brain tissue of patients.[4] Alternatively, as in other repeat-expansion disorders, *C9orf72* mRNA might accumulate in RNA foci, which traps other RNA molecules or RNA binding proteins and thereby affects RNA biology.[4] atg-independent RNA translation has been shown; depending on the frame and the direction in which the repeat is read, the repeat expansion in *C9orf72* encodes several short dipeptide repeat proteins that appear to have toxic properties.[118,119] Dipeptide repeat proteins can be detected in CSF and might be a useful biomarker, either diagnostically or as an outcome measure in clinical trials.

Existing and future treatments

Riluzole is the only widely available drug that prolongs survival of patients with ALS, having been shown in clinical trials to increase median survival from 11·8 months to 14·8 months.[120,121] Edaravone (a free radical scavenger) has been approved for the treatment of ALS in Japan but not elsewhere. Trial outcomes (NCT01492686) that do suggest efficacy remain unpublished, but preliminary reports suggest that edaravone slows functional decline during a 24 week period in a subgroup of patients with recent disease onset and relative preservation of respiratory function.

Nuedexta is effective for treating pseudobulbar affect (uncontrollable laughing or crying), and anecdotal reports claim that nuedexta also improves speech and swallowing.[122] However, nuedexta is not available outside of the USA. Although, initially, marketing authorisation for Europe was granted, this approval was withdrawn at the request of the marketing authorisation holder, apparently on the basis of commercial considerations.

Differences in drug availability and inconsistencies in decisions by regulatory agencies are very frustrating to patients with ALS because they are being denied potentially effective treatments. Approval criteria for treatments for lethal diseases such as ALS between regulatory agencies ought to be harmonised.

Panel: Controversy in amyotrophic lateral sclerosis: genetic testing for all patients?

Notwithstanding the advances in our understanding of amyotrophic lateral sclerosis (ALS) from a genomic perspective, substantial dilemmas remain from a clinical perspective. While some gene mutations in patients with ALS are directly pathogenic, this has not been demonstrated for many reported variants. For instance, more than 150 mutations have been reported in *SOD1*, but irrefutable evidence for direct pathogenicity is only available for a few mutations (eg, Ala5Val, homozygous Asp91Ala).[96,97] Similarly, findings from initial studies suggested that *C9orf72* is fully penetrant by the age of 80 years, but an increasing number of reports of asymptomatic *C9orf72* expansion carriers of advanced age and penetrance estimations using statistical methods suggest that this mutation has only moderate penetrance.[84]

Non-penetrance and genetic pleiotropy in ALS is incompletely understood, and *C9orf72* perhaps best illustrates the complexity of this topic. Disease severity and phenotype seem to be dependent on the size of the repeat expansion (which can vary between cell types within an individual [mosaicism]), methylation status of the promoter, and the expansion itself as well as the presence of genetic variation in other genes (eg, *TMEM106b*, *ATXN2*).[10,98–102]

Genetic counselling to patients with ALS and their relatives is becoming increasingly challenging. There is a growing realisation among patients in the internet era that their disease might be genetic and the patient's right to know is a basic principle of human clinical genetics recognised by most international regulatory statements and legislation.[103,104]

However, given the complexity of the subject, opinions regarding genetic testing differ.[105–107] A group of neurologists and clinical geneticists have proposed guidelines for genetic testing in ALS, with the suggestion that genetic testing should be offered to all patients who have a first or second degree relative with ALS or frontotemporal dementia, and the option of genetic testing should be discussed with all other patients.[105] Counselling should be provided by individuals with an up-to-date understanding of ALS genetics and who are willing to take responsibility for the interpretation of the results. It is advisable to limit testing to those genes for which there is strong evidence for causality, such as *C9orf72*, *TARDBP*, *FUS*, and *SOD1*, and to take into account the local geographic distribution of known causative mutations.[108]

For the **ALS penetrance calculator** see http://alsod.iop.kcl.ac.uk/misc/penetrance.aspx

Precision medicine

ALS is now recognised as a syndrome rather than a single disease entity involving multiple different pathophysiological mechanisms. Although these mechanisms might converge on common pathways, causing recognisable clinical subphenotypes, different ALS-subtypes will probably respond differently to modifying therapies. The greatest challenge in ALS research will be to unravel this heterogeneity and recategorise disease according to genetic subgroup or most relevant pathophysiological feature (figure 4) to facilitate the development of targeted

	Predominant pathology	Associated genes
Classic ALS	TDP-43	*ALS2, SETX, TARDBP, VAPB,CHMP2b, ANG, UBQLN2, OPTN, PFN1, TUBA4a, UNC13a, FIG4, ELP3, NEK1, C21orf2, SIGMAR1, DCTN1, MATR3, CHCHD10, VCP, hnRNPA1, hnRNPA2b1, NIPA1, SMN1, TBK1, ATXN2, MOBP, SARM1, UBQLN2, SQSTM1*
Classic ALS	SOD1	*SOD1*
Classic ALS	FUS	*FUS*
ALS with cognitive or behavioural impairment or comorbid FTD	TDP-43	*TARDBP, CHMP2b, TBK1, UBQLN2, SQSTM1, DCTN1, UNC13a*
Classic ALS, ALS-FTD, FTD	TDP-43, p62, dipeptide repeats, RNA foci	*C9orf72*
Multi-system proteinopathy*	TDP-43	*VCP, hnRNPA1, hnRNPA2b1, SQSTM1*
Behavioural variant FTD	TDP-43	*CHMP2, GRN*
Behavioural variant FTD	FUS	-
Behavioural variant FTD	Tau	*MAPT*
Semantic variant primary progressive aphasia	TDP-43	*GRN, C9orf72*
Semantic variant primary progressive aphasia	Tau	*MAPT*
Logopenic and non-fluent variant primary progressive aphasia	Tau	*MAPT*

ALS=amyotrophic lateral sclerosis. FTD=frontotemporal dementia. *A familial disorder in which patients present with ALS, FTD, inclusion body myositis, Paget's disease of the bone, or combinations thereof.

***Table 3*: The complex correlations between genes, pathology, and phenotypes**

treatments and introduce precision medicine. The way trials are conducted would need to change substantially: inclusion criteria would be based on genetics or other biomarkers, necessitating large-scale international harmonisation of subtype classification to permit enrolment of sufficient numbers of patients.

The first steps towards precision medicine for patients with ALS have already been taken. A successful phase 1 study with *SOD1* antisense oligonucleotides has reached completion, and a new phase 1 trial with a potentially more effective oligo is underway.[123] Many research groups are investigating *C9orf72* antisense oligonucleotides, viral delivery of siRNA, and small molecules as gene-silencing therapies. Initially, neural-specific *C9orf72* knockout in mouse models did not show any phenotype, suggesting that this would be a safe strategy.[124] However, the complete *C9orf72* knockdown in mouse causes severe immune system dysfunction and neoplastic events,[125] so selective knockdown of the expanded allele will be essential.

In a study with patients with Alzheimer's disease,[126] the monoclonal antibody aducanumab was shown to selectively target aggregated Aβ and reduce the concentration of soluble and insoluble Aβ in a dose-dependent manner. Monthly intravenous infusions were also found to slow memory decline in patients with prodromal or mild Alzheimer's disease.[126] One could therefore contemplate targeting TDP-43 in a similar fashion. TDP-43 synthesis is, however, tightly regulated, and overexpression and knock-down could be detrimental and far from straightforward.

Pioneering work with neural stem-cell transplantation into the spinal cord of patients with ALS is revealing that such a procedure can be done safely. Results from efficacy trials are eagerly awaited.[127,128]

Symptomatic therapies

In the absence of effective pharmacological treatments, symptomatic interventions and supportive care remain the cornerstone of ALS management.[129–131] Several of these symptomatic therapies are associated with a clear survival benefit, whereas other therapies provide symptom relief and therefore improve quality of life.

Care is most effective and improves survival when delivered by a multi-disciplinary team of physiotherapists, occupational therapists, speech therapists, respiratory specialists, dietitians, gastroenterologists, social workers, family doctors, neurologists, and rehabilitation specialists.[132,133]

Weight loss is common in ALS and is multifactorial in nature (loss of muscle, hypermetabolism, difficulties eating [swallowing, shortness of breath], or decreased appetite). Prevention of malnutrition improves survival and quality of life.[134] Most guidelines recommend that patients who have lost 10% of bodyweight undergo gastrostomy to enable enteral feeding and to sustain nutrition and medication intake; however, gastrostomy might be most effective at an earlier stage (ie, after 5% weight loss).[135]

Non-invasive ventilation prolongs survival with an effect size greater than riluzole.[136] Nightly non-invasive ventilation (and during daytime if needed) has been shown to increase median survival by 7 months and also improves quality of life;[136] however, its use relies on substantial effort from patients, carers, and respiratory doctors and is therefore not always feasible, particularly for those patients with cognitive or bulbar impairment. Nevertheless, the outcomes of a large cohort study (n=929)[137] suggest that non-invasive ventilation also improves survival in patients with bulbar onset, so all patients should be offered non-invasive ventilation, even when the procedure is likely to be poorly tolerated.

Considering the challenges associated with non-invasive ventilation, alternative strategies for maintaining or supporting respiration are desirable. Diaphragm pacing, or phrenic stimulation, was an approved treatment for respiratory failure on the basis that diaphragm pacing implantation appeared safe and improved survival in patients who had diaphragm pacing implants and received non-invasive ventilation compared with historical controls who received non-invasive ventilation only (37·5 months *vs* 21·4 months).[138,139] However, this finding has been challenged by the outcomes of two recent randomised controlled trials: in both studies,[140,141] mortality was higher in the group of patients who had diaphragm pacing implants and non-invasive ventilation than in the group of patients who received non-invasive ventilation only. As a result,

both trials were stopped prematurely. Although the mechanism underlying a potentially harmful effect of diaphragm pacing is not clear, diaphragmatic pacing is not recommended as a routine treatment for respiratory failure in ALS.

During the course of the disease, many signs and symptoms can develop, such as excess salivation, emotional lability, dropped head, frozen shoulder, pain, cramps, and spasticity. Expert consensus guidelines for the management of these disease aspects are available.[129,130,142]

Biomarkers

The search for reliable biomarkers is a high priority in ALS research.[143] Diagnostic biomarkers could reduce diagnostic delay (9–12 months at present) and would facilitate early initiation of treatment, which is probably when treatment of a neurodegenerative disease is most effective.

Measures of disease progression

The primary outcome measure in ALS trials is survival or rate of decline, or both, on the ALS Functional Rating Scale–revised (ALS-FRS-R).[144,145] Although robust, a considerable amount of time needs to pass before these outcome measures become informative, resulting in lengthy and expensive trials. Early and reliable biomarkers could shorten the duration of trials and make them moree efficient.

Muscle strength and respiratory function have been studied extensively as markers of disease progression. Muscle strength can be measured in several ways.[146–148] Hand-held dynamometry is probably the preferred method because it is easy, cheap, quantitative, reliable, and reproducibly measures decreases in muscle strength in patients with ALS.[149] Various measures exist for respiratory function, including vital capacity, sniff nasal inspiratory pressure, and maximal inspiratory pressure. Differences of opinion exist on which is the best measure, and all are commonly used.

Although muscle strength and respiratory function are informative markers, they do not change early in the disease course. Motor neuron loss is initially compensated for by reinnervation, and clinical weakness only becomes apparent after a substantial number of motor neurons are lost. In most patients, respiratory dysfunction develops late in the disease, so more accurate biomarkers of disease progression are urgently needed. Considering that ALS affects both the lower and upper motor neurons and the frontal and temporal lobes, different biomarkers might be needed for different disease aspects.[150]

Biomarkers of lower motor neuron loss

Loss of lower motor neurons before the development of clinical weakness can be assessed using different electrodiagnostic methods.[151] Data from nerve conduction studies show that the compound muscle action potential amplitude decreases with time and is sensitive to disease progression; however, it is also affected by reinnervation and therefore does not allow quantification of lower motor neuron loss. Motor unit number estimation (MUNE)[152] and Motor Unit Index (MUNIX)[153] are techniques that can be used to measure the number of remaining motor units innervating a muscle. With MUNE, the maximal compound muscle action potential is divided by the mean surface single motor unit action potential, whereas with MUNIX, the number and size of motor unites is derived from the inference pattern on surface electromyography and maximal compound muscle action potential at different grades of voluntary muscle contraction. The advantage of MUNE and MUNIX is that they provide an estimate of the number of motor units, although this also correlates with the reduction in compound muscle action potential. Other potential biomarkers under investigation include nerve excitability, electrical impedance myography, and muscle ultrasound.[154–156] Each technique has its pros and cons with regards to reproducibility, availability, and complexity. At present, there is no single preferred method.

Biomarkers of upper motor neuron loss

Transcranial magnetic stimulation is a non-imaging-based technique to measure upper motor neuron dysfunction. A magnetic coil is used to excite neurons in the underlying motor cortex, and motor-evoked potentials are recorded over a contralateral hand muscle. Transcranial magnetic stimulation improves the sensitivity of ALS diagnosis but is unfortunately technically challenging in patients with severe hand muscle atrophy.[157]

Imaging biomarkers

Loss of upper motor neurons can be difficult to detect clinically when masked by lower motor neuron loss and if no validated clinical upper motor neuron scores are available. Other measures are therefore desirable. Various imaging techniques have been widely applied to study upper motor neuron loss. MRI can be used to distinguish ALS cases from mimics and healthy controls at group level, and some data suggest that thinning of the primary motor cortex is a sensitive diagnostic marker at the individual patient level.[158,159] Diffusion tensor MRI has 65% diagnostic sensitivity and 67% diagnostic specificity for ALS.[160]

^{18}F-FDG-PET has also been proposed as a diagnostic biomarker. It has been reported to detect motor and extra-motor hypometabolism and hypermetabolism in brainstem and medial temporal cortex, with an overall accuracy in discriminating patients with ALS from controls of 93%.[161,162]

Wet biomarkers

Blood or CSF biomarkers would be equally attractive, and the most interesting candidates at present are neurofilaments, which are major structural proteins in

neurons that are released after neuronal damage. The concentration of neurofilament light chain and phosphorylated heavy chain in CSF have good sensitivity (77% and 83%, respectively) and specificity (85% and 77%, respectivey) in differentiating ALS from mimics and show moderate correlation with progression.[163] Serum neurofilament light chain has more than 90% sensitivity and specificity for distinguishing patients with ALS from healthy controls, but no comparisons have been made between patients with ALS and patients with ALS-mimics.[164] Changes in plasma neurofilament light chain concentration correlate with ALS clinical staging, suggesting its potential as a marker for disease progression.[165]

Biomarkers of disease progression

Longitudinal cognitive and behavioural screening could potentially detect changes over time and therefore serve as biomarkers for spread of the disease to other brain areas (frontal and temporal lobes). Considering that TDP-43 aggregation is the pathological hallmark of ALS, it stands to reason that being able to image TDP-43 aggregation in vivo, as is possible with amyloid and Tau, could be a powerful biomarker for all disease aspects, and efforts to this end are underway.[166]

Although these techniques show promise, they all rely on equipment, time, expertise, and substantial resources. The ideal biomarker would be reliable and simple to measure. A potential solution is to measure disease progression through staging, allowing the use of time from one stage to another instead of survival as an outcome measure. Several staging systems exist and indeed correlate with existing measures.[167,168]

Conclusions

ALS is a heterogeneous syndrome that shares pathobiological features with frontotemporal dementia. Rapid gene discovery has facilitated the study of its molecular biology, and many different genetic models of ALS now exist. Studying these disease models has pinpointed potential therapeutic targets, fuelling a sense of optimism that continued efforts will lead to urgently needed treatments for patients with ALS.

Contributors

MAvE and LHvdB did the literature review, coordinated authors' writing, revision, and editing, wrote the first draft, prepared figures, and finalised the manuscript. OH did the literature search and contributed to sections on epidemiology and cognition and was involved in drafting the revision and editing of the final version of the manuscript. AC did the literature search and contributed to sections on epidemiology, cognition, and biomarkers. AA-C did the literature search and contributed to the sections on endogenous retroviruses, genetics, and biomarkers. RJP and JHV did the literature search and contributed to sections on genetics and from genes to biology. All authors were involved in critical revision of the manuscript.

Declaration of interests

MAvE serves on the Motor Neurone Disease Association biomedical research advisory panel, has consulted for Biogen, and has received travel grants from Baxalta; other funding sources include the Netherlands Organization for Health Research and Development (Veni scheme), The Thierry Latran Foundation, and the ALS Foundation Netherlands. LHvdB declares travel grants and consultancy fees from Baxalta and serves on the advisory board for Biogen and Cytokinetics. AA-C serves on the MND Association genetics and epidemiology data access committee and has consulted for Biogen, OrionPharma, Cytokinetics, and Mitsubishi-Tanabe. AC serves on the advisory board for Biogen, Cytokinetics, and Mitsubishi Tanabe. All other authors declare no competing interests.

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