REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive, paralytic disorder characterized by degeneration of motor neurons in the brain and spinal cord. It begins insidiously with focal weakness but spreads relentlessly to involve most muscles, including the diaphragm. Typically, death due to respiratory paralysis occurs in 3 to 5 years.

Motor neurons are grouped into upper populations in the motor cortex and lower populations in the brain stem and spinal cord; lower motor neurons innervate muscle (Fig. 1). When corticospinal (upper) motor neurons fail, muscle stiffness and spasticity result. When lower motor neurons become affected, they initially show excessive electrical irritability, leading to spontaneous muscle twitching (fasciculations); as they degenerate, they lose synaptic connectivity with their target muscles, which then atrophy.

ALS typically begins in the limbs, but about one third of cases are bulbar, heralded by difficulty chewing, speaking, or swallowing. Until late in the disease, ALS spares neurons that innervate the eye and sphincter muscles. The diagnosis is based primarily on clinical examination in conjunction with electromyography, to confirm the extent of denervation, and laboratory testing, to rule out reversible disorders that may resemble ALS.[1,2]

A representative case involves a 55-year-old patient who was evaluated for foot drop, which had begun subtly 4 months earlier with the onset of muscle cramping in the right calf as a result of volitional movement (known as volitional cramping) and had progressed to severe weakness of ankle dorsiflexion and knee extension. In addition to these features, the physical examination revealed atrophy of the right calf and hyperreflexia of the right biceps and of deep tendon reflexes at both knees and both ankles. The neurologic examination was otherwise normal. Electromyography showed evidence of acute muscle denervation (fibrillations) in all four limbs and muscle reinnervation in the right calf (high-amplitude compound muscle action potentials). Imaging of the head and neck revealed no structural lesions impinging on motor tracts, and the results of laboratory studies were normal, findings that ruled out several disorders in the differential diagnosis, such as peripheral neuropathy, Lyme disease, vitamin B_{12} deficiency, thyroid disease, and metal toxicity.[3] A full evaluation disclosed no evidence of a reversible motor neuron disorder, such as multifocal motor neuropathy with conduction block, which is typically associated with autoantibodies (e.g., anti-GM_1 ganglioside antibodies) and can be effectively treated with intravenous immune globulin.[4]

The clinical presentation of ALS is heterogeneous with respect to the populations of involved motor neurons and survival (Fig. 2).[2] When there is prominent involvement of frontopontine motor neurons that serve bulbar functions, a striking finding is emotional lability, indicating pseudobulbar palsy, which is characterized by facial spasticity and a tendency to laugh or cry excessively in response to minor emotional stimuli.

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In primary lateral sclerosis, there is selective involvement of corticospinal and corticopontine motor neurons, with few findings of lower motor neuron dysfunction.[5] Primary lateral sclerosis is ruled out in the representative case described above because of the atrophy and electromyographic findings, which are indicative of lower motor neuron disease. Primary lateral sclerosis progresses slowly, with severe spastic muscle stiffness and little muscle atrophy. This disorder overlaps clinically with a broad category of corticospinal disorders designated as hereditary spastic paraplegias, which are typically symmetrical in onset, slowly progressive, and sometimes associated with sensory loss and other multisystem findings. In primary lateral sclerosis but not hereditary spastic paraplegias, bulbar involvement may be prominent. In progressive muscular atrophy, lower motor neuron involvement is predominant, with little spasticity. The hyperreflexia in the representative case is inconsistent with progressive muscular atrophy.

During the past two decades, it has been recognized that 15 to 20% of persons with ALS have progressive cognitive abnormalities marked by behavioral changes, leading ultimately to dementia.[6] Since these behavioral alterations correlate with autopsy evidence of degeneration of the frontal and temporal lobes, the condition is designated frontotemporal dementia. It was formerly called Pick's disease.

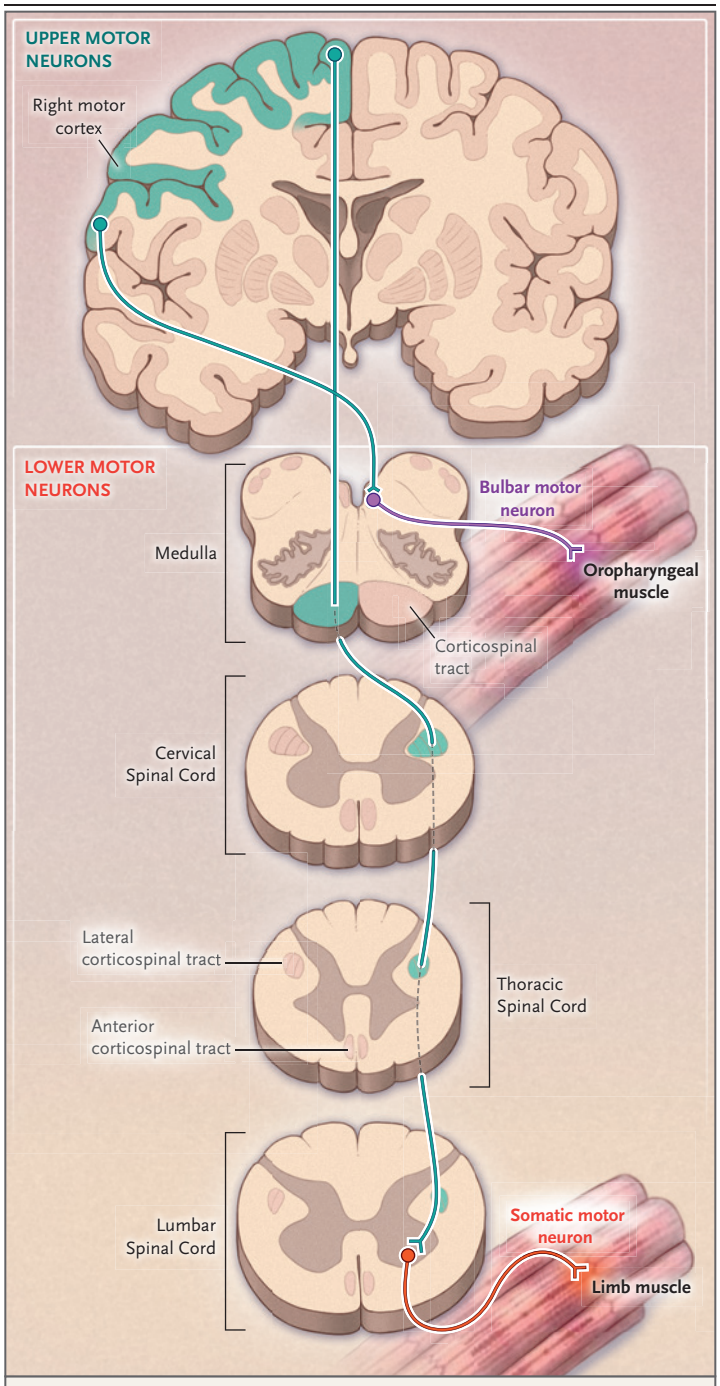

Figure 1. The Motor System.
The motor system is composed of corticospinal (upper) motor neurons in the motor cortex and bulbar and spinal (lower) motor neurons, which innervate skeletal muscle.

EPIDEMIOLOGIC FEATURES

In Europe and the United States, there are 1 or 2 new cases of ALS per year per 100,000 people; the total number of cases is approximately 3 to 5 per 100,000.[7,8] These statistics are globally fairly uniform, although there are rare foci in which ALS is more common. The incidence and prevalence of ALS increase with age. In the United States and Europe, the cumulative lifetime risk of ALS is about 1 in 400; in the United States alone, 800,000 persons who are now alive are expected to die from ALS.[9] About 10% of ALS cases are familial, usually inherited as dominant traits.[10] The remaining 90% of cases of ALS are sporadic (occurring without a family history). In cases of sporadic ALS, the ratio of affected males to affected females may approach 2:1; in familial ALS, the ratio is closer to 1:1. ALS is the most frequent neurodegenerative disorder of midlife, with an onset in the middle-to-late 50s. An onset in the late teenage or early adult years is usually indicative of familial ALS. The time

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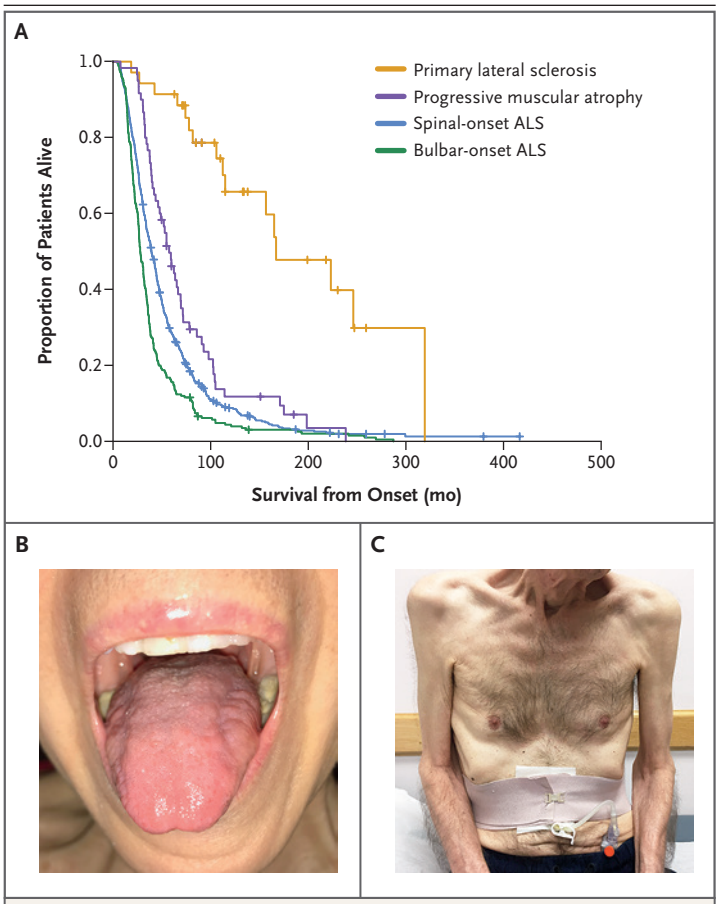

Figure 2. Phenotype and Survival in Amyotrophic Lateral Sclerosis (ALS). Panel A shows survival curves for two types of ALS (spinal-onset and bulbar-onset) and two other motor neuron diseases (primary lateral sclerosis and progressive muscular atrophy). Panel B shows lateral atrophy and furrowing of the tongue in a patient with ALS, findings that reflect denervation due to degeneration of bulbar motor neurons. Panel C shows thinned arms and shoulders, findings that are typical of the flail-arm syndrome, which occurs in patients with ALS and is associated with protracted survival.

from the first symptom of ALS to diagnosis is approximately 12 months, a problematic delay if successful therapy requires early intervention. Because an abundance of ALS genes have now been identified, it will probably be informative to reanalyze this epidemiologic profile of ALS with stratification according to genetically defined subtypes.

PATHOLOGICAL CHARACTERISTICS

The core pathological finding in ALS is motor neuron death in the motor cortex and spinal cord; in ALS with frontotemporal dementia, neuronal degeneration is more widespread, occurring throughout the frontal and temporal lobes. Degeneration of the corticospinal axons causes thinning and scarring (sclerosis) of the lateral aspects of the spinal cord. In addition, as the brain stem and spinal motor neurons die, there is thinning of the ventral roots and denervational atrophy (amyotrophy) of the muscles of the tongue, oropharynx, and limbs. Until late in the disease, ALS does not affect neurons that innervate eye muscles or the bladder. Degeneration of motor neurons is accompanied by neuroinflammatory processes, with proliferation of astroglia, microglia, and oligodendroglial cells.[11,12] A common feature in cases of both familial and sporadic ALS is aggregation of cytoplasmic proteins, prominently but not exclusively in motor neurons. Some of these proteins are common in most types of ALS. This is exemplified by the nuclear TAR DNA-binding protein 43 (TDP-43), which in many cases of ALS is cleaved, hyperphosphorylated, and mislocalized to the cytoplasm.[13] Aggregates of ubiquilin 2 are also common,[14] as are intracytoplasmic deposits of wild-type superoxide dismutase 1 (SOD1) in sporadic ALS.[15] Many protein deposits show evidence of ubiquitination; threads of ubiquitinated TDP-43 are prominent in motor neurons, both terminally and before atrophy of the cell body. Given the diverse causes of ALS, it is not surprising that some types of aggregates are detected only in specific ALS subtypes (e.g., dipeptide aggregates and intranuclear RNA deposits in *C9ORF72* ALS).

GENETIC FEATURES

Evolving technologies for gene mapping and DNA analysis have facilitated the identification of multiple ALS genes (Fig. 3). *SOD1* was the first ALS gene to be identified, in 1993.[16] More than 120 genetic variants have been associated with a risk of ALS[17] (http://alsod.iop.kcl.ac.uk). Several criteria assist in identifying those that are most meaningful. The strongest confirmation is validation in multiple independent families and cohorts. Also supportive are an increased burden of the variant in cases relative to controls and the predicted consequences of the variant (e.g., missense mutation vs. truncation). It has proved almost impossible to predict a variant's relevance to ALS from the biologic features of the gene itself. As shown in Figure 3, at least 25

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genes have now been reproducibly implicated in familial ALS, sporadic ALS, or both.[18-20]

A by-product of the genetic studies that is highly relevant to therapeutic development has been the generation of mouse models of ALS. Strikingly, transgenic expression of mutant SOD1 protein[21] and, more recently, profilin 1 (PFN1)[22] generates a neurodegenerative, paralytic process in mice that mimics many aspects of human ALS. An important lesson from transgenic models of TDP-43 and FUS (fused in sarcoma) is that levels of the normal protein are tightly controlled. In contrast with SOD1, forced expression of high levels of normal TDP-43 by itself triggers motor neuron degeneration.[23] Mouse models of *C9orf72* (the 72nd open reading frame identified on chromosome 9, the most commonly mutated gene in ALS) have now also been generated for *C9ORF72* ALS and are discussed below.

Correlations between genetic variants and different clinical profiles in ALS, such as age at onset, disease duration, and site of onset, have been defined (Table 1). An important example is the gene that encodes the enzyme ephrin A4 (*EPHA4*)[33] — lower levels of expression of *EPHA4* correlate with longer survival. Some genetic variants influence both susceptibility and phenotype. For example, progression is accelerated in patients with the common A4V mutation[30] of *SOD1* and in patients with the P525L mutation of *FUS/TLS*; the latter may lead to fulminant, childhood-onset motor neuron disease.[28]

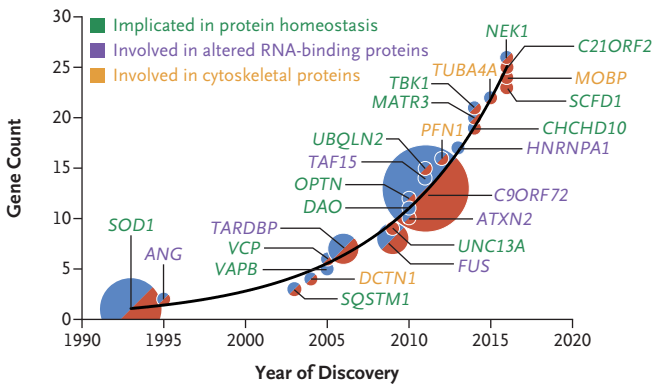

Figure 3. ALS Gene Discovery since 1990.

The cumulative numbers of known ALS genes have increased rapidly. The size of each circle reflects the proportion of all familial ALS cases associated with that gene (e.g., 20% for *SOD1* and 45% for *C9ORF72*). Blue circles indicate genes associated only with familial ALS, red circles indicate genes associated only with sporadic ALS, and circles that are half blue and half red indicate genes associated with both familial and sporadic ALS. Each of these genes has been found to be mutated in more than one ALS-affected family or in multiple, unrelated cases of sporadic ALS.

CONCEPTS IN PATHOGENESIS

A comprehensive explanation for ALS must include both its familial and sporadic forms, as well as categories of phenotypic divergence that arise even with the same proximal trigger, such as a gene mutation. A general presumption has been that the disease reflects an adverse interplay between genetic and environmental factors. An alternative view postulates that all cases of ALS are a consequence primarily of complex genetic factors. Several perspectives suggest that the pathogenesis of ALS entails a multistep process.[34]

LESSONS FROM FAMILIAL ALS

There is striking heterogeneity in the genetic causes of familial ALS, but familial ALS and sporadic ALS have similarities in their pathological features, as well as in their clinical features, suggesting a convergence of the cellular and molecular events that lead to motor neuron degeneration. These points of convergence define targets for therapy.

A working view of the present panel of ALS genes is that they cluster in three categories,[19] involving protein homeostasis, RNA homeostasis and trafficking, and cytoskeletal dynamics (Fig. 4). These mechanisms are not exclusive. For example, protein aggregates may sequester proteins that are important in RNA binding, thereby perturbing RNA trafficking and homeostasis. Moreover, these mechanisms are detected in the context of both familial ALS and sporadic ALS; some nonmutant proteins also have a propensity to misfold and aggregate in ALS, much like their mutant counterparts (e.g., SOD1 and TDP-43).

Downstream of each category are diverse forms of cellular abnormalities, including the deposition of intranuclear and cytosolic protein and RNA aggregates, disturbances of protein degradative mechanisms, mitochondrial dysfunction, endoplasmic reticulum stress, defective nucleocytoplasmic trafficking, altered neuronal excitability, and altered axonal transport. In most cases, these events activate and recruit nonneuronal cells (astrocytes, microglia, and oligodendroglia), which exert both salutary and

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Table 1. Genetic Variants That Influence the Phenotype in Amyotrophic Lateral Sclerosis.

Gene	Minor Allele Frequency or Expression Level	Phenotype			Study
		Site of Onset	Effect of Minor Allele on Age at Onset*	Effect of Minor Allele on Survival†	
Genomewide association study					
rs3011225-1p34	0.22		2 yr later		Ahmeti et al.[24]
UNC13A	0.40			Shorter by 5–10 mo	Diekstra et al.[25]
CAMTA1	0.26			Shorter by about 5 mo	Fogh et al.[26]
IDE	0.03			Shorter by about 7 mo	Fogh et al.[26]
Known ALS genes					
C9ORF72	Up to 0.08	Primarily bulbar			Cooper-Knock et al.[27]
FUS-P525L	Rare variation		Many years earlier	Shorter by several months	Conte et al.[28]
PFN1	Rare variation	Limb			Wu et al.[29]
SOD1-A4V	Rare variation	Limb		Shorter by several months	Cudkowicz et al.[30]
SOD1/SOD1	Rare variation		Many years earlier	Shorter by several months	Winter et al.[31]
Modifier genes					
APOE	Expression increased			Longer by several months	Lacomblez et al.[32]
EPHA4	Expression decreased			Longer by several months	Van Hoecke et al.[33]

* The effect of the minor allele on age is shown relative to a cohort with the major allele.
† The effect of the minor allele on survival is shown relative to a cohort with the major allele.

negative influences on motor neuron viability. The diverse downstream abnormalities may differentially affect subcellular compartments (dendrites, soma, axons, and neuromuscular junctions). One implication of this model is that successful therapy for ALS will require simultaneous interventions in multiple downstream pathways.

GENES THAT INFLUENCE PROTEIN HOMOEOSTASIS

The most extensively investigated pathological finding in ALS has been the accumulation of aggregated proteins and corresponding defects in the cellular pathways for protein degradation. Mutant SOD1 frequently forms intracellular aggregates. Genes that encode adapter proteins involved in protein maintenance and degradation are also implicated in ALS. These include valosin-containing protein (VCP)[35] and the proteins optineurin (OPTN),[36] TANK-binding kinase 1 (TBK1),[37-39] and sequestosome 1 (SQSTM1/p62)[40] (Fig. 4A). The TBK1–OPTN axis is interwoven in other neurodegenerative disorders; for example, the Parkinson's disease gene *PINK1* encodes a protein that acts upstream of TBK1 in the mobilization of mitophagy.

GENES THAT INFLUENCE RNA HOMEOSTASIS AND TRAFFICKING

The most rapidly expanding category of ALS genes encodes proteins that interact with RNA. The first protein to be discovered was TDP-43,[13] whose mislocalization from the nucleus to the cytosol, cleavage, phosphorylation, and ubiquitination were initially illuminated in sporadic ALS and frontotemporal dementia. However, it became apparent that mutations in *TARDBP*, the gene encoding TDP-43, can cause familial ALS.[41] Mislocalization and post-translational modification of TDP-43 are observed in many neurode-

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generative diseases. *FUS-TLS* encodes another RNA-binding protein, homologous to TDP-43, which in mutant form also causes ALS.[42,43] Why mutated genes encoding RNA-binding proteins cause ALS is not clear. These proteins have multiple functions in gene splicing, surveillance of transcripts after splicing, generation of microRNA, and axonal biologic processes. Most of these proteins have low-complexity domains that permit promiscuous binding not only to RNA but also to other proteins. The ALS-related mutations heighten this binding propensity, leading to self-assembly of the proteins and the formation of aggregates.[44] This auto-aggregation is facilitated in stress granules, which are non–membrane-bound structures formed under cell stress that contain RNA complexes stalled in translation.[45-47] The self-assembly of mutant RNA-binding proteins may induce toxic, self-propagating conformations that disseminate disease within and between cells in a manner analogous to that of prion proteins.

The most commonly mutated gene in ALS is *C9ORF72*.[48-50] The C9ORF72 protein has a role in nuclear and endosomal membrane trafficking and autophagy. A noncoding stretch of six nucleotides is repeated up to approximately 30 times in normal persons. Expansions of this segment to hundreds or thousands of repeats cause familial ALS and frontotemporal dementia; in addition, these expansions sometimes cause sporadic ALS. Several mechanisms may contribute to the neurotoxicity of the hexanucleotide expansion (Fig. 4B). Transcripts of the offending segments are deposited in the nucleus, forming RNA foci that sequester nuclear proteins. Some of the expanded RNA escapes to the cytoplasm, where it generates five potentially toxic repeat dipeptides through a noncanonical translation process. Recent studies have also shown a defect in transport across the nuclear membrane in cells with the *C9ORF72* expansions.[51,52] A reduction in the total levels of the normal C9ORF72 protein may also contribute to neurotoxicity.[53-55] Transgenic mouse models of *C9orf72* recapitulate the molecular features of *C9ORF72* ALS in humans[56-59] but, with one exception,[59] do not show a strong motor phenotype.

GENES THAT INFLUENCE CYTOSKELETAL DYNAMICS

Three ALS genes encode proteins that are important in maintenance of normal cytoskeletal dynamics: dynactin 1 (DCTN1),[60] PFN1,[29] and tubulin 4A (TUBA4A) (Fig. 4C).[61] TUBA4A dimers are components of microtubules, whose integrity is essential for axonal structure; DCTN1 is implicated in retrograde axonal transport, whereas PFN1 participates in the conversion of globular to filamentous actin and nerve extension. Also implicated is the modifier gene *EPHA4*; lower levels of *EPHA4* expression correlate with longer survival in ALS, perhaps because they permit more exuberant axonal extension.

INSIGHTS INTO SPORADIC ALS

Despite the absence of a family history in sporadic ALS, studies involving twins show that the heritability is about 60%.[62] Furthermore, mutations usually found in familial ALS can be found in sporadic ALS. This can be partly explained by the difficulty in ascertaining whether patients with late-onset disease have a family history of ALS. The situation is confounded by the observation that some familial ALS gene variants increase the risk of phenotypes other than ALS, such as frontotemporal dementia.[38,39,48] Unless these other phenotypes are recognized as relevant, the family history may be incorrectly recorded as negative. In addition, several familial ALS gene variants are of intermediate penetrance (e.g., the *C9ORF72* hexanucleotide repeat expansion, *ATXN2* repeat expansions,[63] and *TBK1* mutations).[37-39] Thus, ALS might not be manifested in a gene carrier, in which case, the disease is characterized by familial clustering rather than mendelian inheritance and may appear to be sporadic.[64] Combinations of such gene variants further increase the risk of ALS and may be another cause of apparently sporadic ALS.[65]

Recent genomewide association studies have shown that rare genetic variation is disproportionately frequent in sporadic ALS.[66] The genetic architecture of sporadic ALS is markedly different from that of complex diseases such as schizophrenia in which there are additive effects of hundreds of common variants, each with a minute effect on risk. However, common variants still have a part to play in sporadic ALS. For example, variants in the genes *UNC13A*, *MOBP*, and *SCFD1* all increase the risk by a small but significant degree.[66]

Heritability studies also show that a substantial fraction of cases of sporadic ALS cannot be attributed to genetic or biologic factors; these

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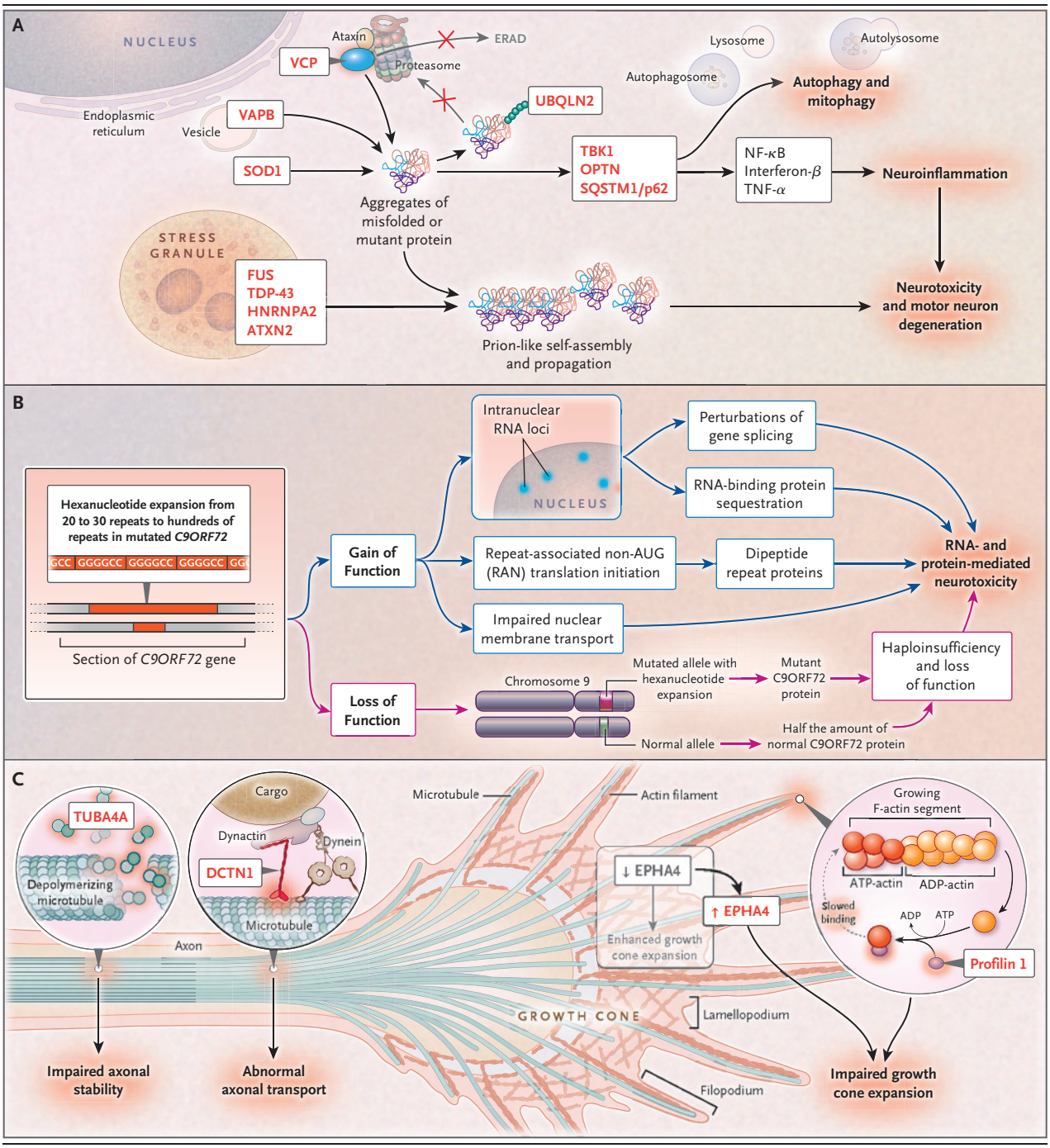

cases are ascribed to environmental or undefined factors. Attempts to identify occupations or common exposures that might increase the risk of ALS have been inconclusive. Environmental studies are challenging because the number of possible exposures is large, and a critical, disease-related exposure may have happened many years before the onset of the disease. A particular difficulty is that studies of ALS are susceptible to bias because of the poor prognosis. Patients who live long enough to attend a specialist research clinic are different from those identified in population studies, and this difference can cause bias in the results. For instance, smok-

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Figure 4 (facing page). Three Major Categories of Pathophysiological Processes in ALS.

The pathways relating the implicated proteins (red) and key cellular structures and molecules (gray) are shown. Downstream dysfunctional events are black within gray boxes. Panel A shows altered protein homeostasis in ALS. Many ALS genes encode adapter proteins that are critical in protein degradation, acting at the level of the endoplasmic reticulum (endoplasmic reticulum–associated protein degradation [ERAD]) and through proteosomal and autophagic pathways. RNA-binding proteins may self-assemble to form prion-like aggregates. Panel B shows mechanisms of *C9ORF72*-related disease. The toxicity of expanded hexanucleotide repeats in the *C9ORF72* gene is proposed to involve depositions of intranuclear RNA, with resulting perturbations of gene splicing and sequestration of RNA-binding proteins; noncanonical translation of polydipeptides from the expanded DNA, yielding toxic repeat dipeptides; disturbances of nucleocytoplasmic transport; and reduced levels of C9ORF72 (haploinsufficiency). Panel C shows altered neuronal cytoskeletal dynamics in ALS. Genes encoding dynactin (*DCTN1*) and tubulin 4A (*TUBA4A*) are essential in the maintenance of the structure of the motor nerve axon; mutations in these genes disturb both axonal integrity and axonal transport. Profilin 1 (PFN1) is essential for the assembly of filamentous axons and the formation of distal axonal growth cones. *PFN1* mutations and increased expression of ephrin A4 (EPHA4) slow the extension of the distal axon. ADP denotes adenosine diphosphate, NF-κB nuclear factor kappa light-chain enhancer of activated B cells, and TNF tumor necrosis factor.

ing has been shown to shorten survival in a population study,[67] so a case–control study selecting participants from clinics would find smokers underrepresented in the ALS group and would thus suggest that smoking either has no effect or might be protective. Similarly, ALS specialists report anecdotally that their patients tend to be athletic, slim, and very fit,[68] but if these factors slow disease progression rather than increase risk, such patients will be overrepresented at specialist centers.

Notwithstanding the barriers to identifying environmental risk factors, some factors have been associated with ALS in multiple studies.[69,70] The exposure with the strongest support is military service.[71,72] In addition, smoking has been implicated as a dose-dependent risk factor for ALS.[73] Exposure to heavy metals may be important; blood lead levels and cerebrospinal fluid manganese levels are higher in patients with ALS than in controls.[70] People with occupations involving exposure to electromagnetic fields also appear to be at increased risk, but people living near power lines are not. Other risk factors with varying levels of support include pesticide exposure and neurotoxins such as those produced by cyanobacteria. Viruses have been studied as a possible explanation for sporadic ALS. Initial studies suggesting the role of an activated, endogenous retrovirus[74] were followed by the identification of a possible candidate, human endogenous retrovirus K.[75]

There is increasing evidence that trauma precedes some individual cases of ALS.[76] A meta-analysis has suggested that trauma overall, trauma occurring more than 5 years previously, bone fracture, and head injury are all associated with an increased risk.[77] In recent years, it has been observed that persons engaged in sports that entail repetitive concussions or subconcussive head trauma are at increased risk for ALS and a concurrent behavioral disorder marked by impulsivity and memory loss. Autopsy studies in persons with this disorder, called chronic traumatic encephalopathy, have revealed frontotemporal atrophy associated with distinctive deposits of tau protein, as well as TDP-43, the characteristic inclusion protein in ALS.[78]

THERAPEUTICS AND BEYOND

No therapy offers a substantial clinical benefit for patients with ALS. The drugs riluzole[79] and edaravone, which have been approved by the Food and Drug Administration for the treatment of ALS, provide a limited improvement in survival. Riluzole acts by suppressing excessive motor neuron firing, and edaravone by suppressing oxidative stress. Numerous other compounds that have been investigated have not been shown to be effective.[80,81] Currently, the mainstay of care for patients with ALS is timely intervention to manage symptoms, including use of nasogastric feeding, prevention of aspiration (control of salivary secretions and use of cough-assist devices), and provision of ventilatory support (usually with bilevel positive airway pressure). Some interventions raise serious ethical issues, such as whether to perform tracheostomy for full ventilation and, if so, when and how to withdraw respiratory support once it has been instituted.

Despite the pipeline of potential treatments for ALS, reflecting the expanded list of targets

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identified through genetic studies and increasing numbers of ALS investigators, many of whom are in the pharmaceutical sector,[80,82] no drugs are being investigated in late-phase clinical trials. Several innovative approaches to treating ALS (and other neurodegenerative diseases) are in development. Two examples include the use of adeno-associated viruses (AAV) to achieve widespread delivery of diverse cargoes (missing genes, therapeutic genes, or gene-silencing elements) to the central nervous system and the use of stem cells that provide neurotrophic factors to the central nervous system.[83] Studies in cells, mice, and humans support the view that several types of reagents (e.g., antisense oligonucleotides and AAV-delivered microRNA) inactivate production of toxic gene products and thus may be therapeutic in ALS mediated by genes such as *SOD1*[84-87] and *C9ORF72*. Indeed, clinical trials investigating the use of antisense oligonucleotides to silence *SOD1* have begun.

One can anticipate continued progress in understanding the biology of ALS. There is no doubt that high-throughput genetics, combined with improved clinical phenotyping, will further refine the genetic landscape of ALS. As thousands of full genome sequences become available, it will be feasible to explore the possibility that complex interactions among multiple gene variants explain not only familial ALS but also sporadic ALS. The exploration of environmental factors in sporadic ALS will expand, with a focus on the internal environment represented by the microbiome. The ultimate proof of our understanding of the biology of ALS will hinge on our ability to modify the clinical course of the disease.

Dr. Brown reports holding equity in AviTx, Amylyx Pharmaceuticals, and ImStar Therapeutics, receiving fees for serving on an advisory board from Voyager Therapeutics, negotiating a collaborative agreement with WAVE Biosciences, holding patents and receiving royalties for patents on "Method for the diagnosis of familial amyotrophic lateral sclerosis" (US 5,843,641) and "Mice having a mutant SOD1 encoding transgene" (US 6,723,893), holding a patent for "Compounds and method for the diagnosis, treatment and prevention of cell death" (US 5,849,290), and holding a pending patent for "Use of synthetic microRNA for AAV-mediated silencing of SOD1 in ALS"; and Dr. Al-Chalabi reports receiving consulting fees from GlaxoSmithKline, providing unpaid consulting for Mitsubishi Tanabe Pharma, Treeway, Chronos Therapeutics, and Avanir Pharmaceuticals, receiving consulting fees and serving as principal investigator in an international commercial clinical trial of tirasemtiv in ALS for Cytokinetics, and serving as chief investigator of an international commercial clinical trial of levosimendan in ALS for Orion Pharma. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES

1. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med 2001;344:1688-700.
2. Al-Chalabi A, Hardiman O, Kiernan MC, Chiò A, Rix-Brooks B, van den Berg LH. Amyotrophic lateral sclerosis: moving towards a new classification system. Lancet Neurol 2016;15:1182-94.
3. Rezania K, Roos RP. Spinal cord: motor neuron diseases. Neurol Clin 2013;31:219-39.
4. Arcila-Londono X, Lewis RA. Multifocal motor neuropathy. Handb Clin Neurol 2013;115:429-42.
5. Gordon PH, Cheng B, Katz IB, et al. The natural history of primary lateral sclerosis. Neurology 2006;66:647-53.
6. Bang J, Spina S, Miller BL. Frontotemporal dementia. Lancet 2015;386:1672-82.
7. Robberecht W, Philips T. The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci 2013;14:248-64.
8. Chiò A, Logroscino G, Traynor BJ, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology 2013;41:118-30.
9. Johnston CA, Stanton BR, Turner MR, et al. Amyotrophic lateral sclerosis in an urban setting: a population based study of inner city London. J Neurol 2006;253:1642-3.
10. Mulder DW, Kurland LT, Offord KP, Beard CM. Familial adult motor neuron disease: amyotrophic lateral sclerosis. Neurology 1986;36:511-7.
11. Philips T, Rothstein JD. Glial cells in amyotrophic lateral sclerosis. Exp Neurol 2014;262 Pt B:111-20.
12. Kang SH, Li Y, Fukaya M, et al. Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. Nat Neurosci 2013;16:571-9.
13. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314:130-3.
14. Deng HX, Chen W, Hong ST, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 2011;477:211-5.
15. Bosco DA, Morfini G, Karabacak NM, et al. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat Neurosci 2010;13:1396-403.
16. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993;362:59-62.
17. Taylor JP, Brown RH Jr, Cleveland DW. Decoding ALS: from genes to mechanism. Nature 2016;539:197-206.
18. Therrien M, Dion PA, Rouleau GA. ALS: recent developments from genetics studies. Curr Neurol Neurosci Rep 2016;16:59-71.
19. Peters OM, Ghasemi M, Brown RH Jr. Emerging mechanisms of molecular pathology in ALS. J Clin Invest 2015;125:2548.
20. Pottier C, Ravenscroft TA, Sanchez-Contreras M, Rademakers R. Genetics of FTLD: overview and what else we can expect from genetic studies. J Neurochem 2016;138:Suppl 1:32-53.
21. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 1994;264:1772-5.
22. Yang C, Danielson EW, Qiao T, et al. Mutant PFN1 causes ALS phenotypes and progressive motor neuron degeneration in mice by a gain of toxicity. Proc Natl Acad Sci U S A 2016;113:E6209-E6218.
23. Mitchell JC, Constable R, So E, et al. Wild type human TDP-43 potentiates ALS-

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linked mutant TDP-43 driven progressive motor and cortical neuron degeneration with pathological features of ALS. Acta Neuropathol Commun 2015;3:36.
24. Ahmeti KB, Ajroud-Driss S, Al-Chalabi A, et al. Age of onset of amyotrophic lateral sclerosis is modulated by a locus on 1p34.1. Neurobiol Aging 2013;34(1):357.e7-19.
25. Diekstra FP, Van Deerlin VM, van Swieten JC, et al. C9orf72 and UNC13A are shared risk loci for amyotrophic lateral sclerosis and frontotemporal dementia: a genome-wide meta-analysis. Ann Neurol 2014;76:120-33.
26. Fogh I, Lin K, Tiloca C, et al. Association of a locus in the *CAMTA1* gene with survival in patients with sporadic amyotrophic lateral sclerosis. JAMA Neurol 2016;73:812-20.
27. Cooper-Knock J, Shaw PJ, Kirby J. The widening spectrum of C9ORF72-related disease; genotype/phenotype correlations and potential modifiers of clinical phenotype. Acta Neuropathol 2014;127:333-45.
28. Conte A, Lattante S, Zollino M, et al. P525L FUS mutation is consistently associated with a severe form of juvenile amyotrophic lateral sclerosis. Neuromuscul Disord 2012;22:73-5.
29. Wu CH, Fallini C, Ticozzi N, et al. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature 2012;488:499-503.
30. Cudkowicz ME, McKenna-Yasek D, Sapp PE, et al. Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Ann Neurol 1997;41:210-21.
31. Winter SM, Claus A, Oberwittler C, Völkel H, Wenzler S, Ludolph AC. Recessively inherited amyotrophic lateral sclerosis: a Germany family with the D90A CuZn-SOD mutation. J Neurol 2000;247:783-6.
32. Lacomblez L, Doppler V, Beucler I, et al. APOE: a potential marker of disease progression in ALS. Neurology 2002;58:1112-4.
33. Van Hoecke A, Schoonaert L, Lemmens R, et al. EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans. Nat Med 2012;18:1418-22.
34. Al-Chalabi A, Calvo A, Chio A, et al. Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study. Lancet Neurol 2014;13:1108-13.
35. Watts GD, Thomasova D, Ramdeen SK, et al. Novel VCP mutations in inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. Clin Genet 2007;72:420-6.
36. Maruyama H, Morino H, Ito H, et al. Mutations of optineurin in amyotrophic lateral sclerosis. Nature 2010;465:223-6.
37. Cirulli ET, Lasseigne BN, Petrovski S, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science 2015;347:1436-41.
38. Freischmidt A, Wieland T, Richter B, et al. Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. Nat Neurosci 2015;18:631-6.
39. Pottier C, Bieniek KF, Finch N, et al. Whole-genome sequencing reveals important role for TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease. Acta Neuropathol 2015;130:77-92.
40. Fecto F, Yan J, Vemula SP, et al. SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch Neurol 2011;68:1440-6.
41. Sreedharan J, Blair IP, Tripathi VB, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008;319:1668-72.
42. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 2009;323:1205-8.
43. Vance C, Rogelj B, Hortobágyi T, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 2009;323:1208-11.
44. Kim HJ, Kim NC, Wang YD, et al. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. Nature 2013;495:467-73.
45. Bosco DA, Lemay N, Ko HK, et al. Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. Hum Mol Genet 2010;19:4160-75.
46. Li YR, King OD, Shorter J, Gitler AD. Stress granules as crucibles of ALS pathogenesis. J Cell Biol 2013;201:361-72.
47. Hyman AA, Weber CA, Jülicher F. Liquid-liquid phase separation in biology. Annu Rev Cell Dev Biol 2014;30:39-58.
48. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011;72:245-56.
49. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011;72:257-68.
50. Gijselinck I, Van Langenhove T, van der Zee J, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. Lancet Neurol 2012;11:54-65.
51. Freibaum BD, Lu Y, Lopez-Gonzalez R, et al. GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. Nature 2015;525:129-33.
52. Zhang K, Donnelly CJ, Haeusler AR, et al. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. Nature 2015;525:56-61.
53. Donnelly CJ, Zhang PW, Pham JT, et al. RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. Neuron 2013;80:415-28.
54. Lagier-Tourenne C, Baughn M, Rigo F, et al. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc Natl Acad Sci U S A 2013;110:E4530-E4539.
55. Sareen D, O'Rourke JG, Meera P, et al. Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. Sci Transl Med 2013;5:208ra149.
56. O'Rourke JG, Bogdanik L, Muhammad AK, et al. C9orf72 BAC transgenic mice display typical pathologic features of ALS/FTD. Neuron 2015;88:892-901.
57. Peters OM, Cabrera GT, Tran H, et al. Human C9ORF72 hexanucleotide expansion reproduces RNA foci and dipeptide repeat proteins but not neurodegeneration in BAC transgenic mice. Neuron 2015;88:902-9.
58. Jiang J, Zhu Q, Gendron TF, et al. Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. Neuron 2016;90:535-50.
59. Liu Y, Pattamatta A, Zu T, et al. C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. Neuron 2016;90:521-34.
60. Puls I, Jonnakuty C, LaMonte BH, et al. Mutant dynactin in motor neuron disease. Nat Genet 2003;33:455-6.
61. Pensato V, Tiloca C, Corrado L, et al. TUBA4A gene analysis in sporadic amyotrophic lateral sclerosis: identification of novel mutations. J Neurol 2015;262:1376-8.
62. Al-Chalabi A, Fang F, Hanby MF, et al. An estimate of amyotrophic lateral sclerosis heritability using twin data. J Neurol Neurosurg Psychiatry 2010;81:1324-6.
63. Elden AC, Kim HJ, Hart MP, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 2010;466:1069-75.
64. Al-Chalabi A, Lewis CM. Modelling the effects of penetrance and family size on rates of sporadic and familial disease. Hum Hered 2011;71:281-8.
65. van Blitterswijk M, van Es MA, Hennekam EA, et al. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. Hum Mol Genet 2012;21:3776-84.
66. van Rheenen W, Shatunov A, Dekker AM, et al. Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. Nat Genet 2016;48:1043-8.
67. Calvo A, Canosa A, Bertuzzo D, et al. Influence of cigarette smoking on ALS outcome: a population-based study. J Neurol Neurosurg Psychiatry 2016;87:1229-33.

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68. Lacorte E, Ferrigno L, Leoncini E, Corbo M, Boccia S, Vanacore N. Physical activity, and physical activity related to sports, leisure and occupational activity as risk factors for ALS: a systematic review. Neurosci Biobehav Rev 2016;66:61-79.
69. Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. Nat Rev Neurol 2013;9:617-28.
70. Ingre C, Roos PM, Piehl F, Kamel F, Fang F. Risk factors for amyotrophic lateral sclerosis. Clin Epidemiol 2015;7:181-93.
71. Weisskopf MG, O'Reilly EJ, McCullough ML, et al. Prospective study of military service and mortality from ALS. Neurology 2005;64:32-7.
72. Beard JD, Engel LS, Richardson DB, et al. Military service, deployments, and exposures in relation to amyotrophic lateral sclerosis etiology. Environ Int 2016;91:104-15.
73. Wang H, O'Reilly EJ, Weisskopf MG, et al. Smoking and risk of amyotrophic lateral sclerosis: a pooled analysis of 5 prospective cohorts. Arch Neurol 2011;68:207-13.
74. McCormick AL, Brown RH Jr, Cudkowicz ME, Al-Chalabi A, Garson JA. Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate. Neurology 2008;70:278-83.
75. Li W, Lee MH, Henderson L, et al. Human endogenous retrovirus-K contributes to motor neuron disease. Sci Transl Med 2015;7:307ra153.
76. Seals RM, Hansen J, Gredal O, Weisskopf MG. Physical trauma and amyotrophic lateral sclerosis: a population-based study using Danish national registries. Am J Epidemiol 2016;183:294-301.
77. Wang MD, Little J, Gomes J, Cashman NR, Krewski D. Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis. Neurotoxicology 2016 July 1 (Epub ahead of print).
78. McKee AC, Stein TD, Kiernan PT, Alvarez VE. The neuropathology of chronic traumatic encephalopathy. Brain Pathol 2015;25:350-64.
79. Bensimon G, Lacomblez L, Meininger V, ALS/Riluzole Study Group. A controlled trial of riluzole in amyotrophic lateral sclerosis. N Engl J Med 1994;330:585-91.
80. Beghi E, Chiò A, Couratier P, et al. The epidemiology and treatment of ALS: focus on the heterogeneity of the disease and critical appraisal of therapeutic trials. Amyotroph Lateral Scler 2011;12:1-10.
81. Nicholson KA, Cudkowicz ME, Berry JD. Clinical trial designs in amyotrophic lateral sclerosis: does one design fit all? Neurotherapeutics 2015;12:376-83.
82. Cudkowicz ME, van den Berg LH, Shefner JM, et al. Dexpramipexole versus placebo for patients with amyotrophic lateral sclerosis (EMPOWER): a randomised, double-blind, phase 3 trial. Lancet Neurol 2013;12:1059-67.
83. Glass JD, Hertzberg VS, Boulis NM, et al. Transplantation of spinal cord-derived neural stem cells for ALS: analysis of phase 1 and 2 trials. Neurology 2016;87:392-400.
84. Ralph GS, Radcliffe PA, Day DM, et al. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. Nat Med 2005;11:429-33.
85. Stoica L, Todeasa SH, Cabrera GT, et al. Adeno-associated virus-delivered artificial microRNA extends survival and delays paralysis in an amyotrophic lateral sclerosis mouse model. Ann Neurol 2016;79:687-700.
86. Smith RA, Miller TM, Yamanaka K, et al. Antisense oligonucleotide therapy for neurodegenerative disease. J Clin Invest 2006;116:2290-6.
87. Miller TM, Pestronk A, David W, et al. An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. Lancet Neurol 2013;12:435-42.

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