Amyotrophic Lateral Sclerosis


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 B12 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-GMI 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.
In primary lateral sclerosis, there is selective involvement of corticospinal and corticopontine motor neurons, with few findings of lower motor neuron dysfunction. 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. 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.

**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. 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. About 10% of ALS cases are familial, usually inherited as dominant traits. 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...
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 ALS17 (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
genes have now been reproducibly implicated in familial ALS, sporadic ALS, or both.\textsuperscript{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\textsuperscript{21} and, more recently, profilin 1 (PFN1)\textsuperscript{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.\textsuperscript{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)\textsuperscript{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\textsuperscript{10} of SOD1 and in patients with the P52SL mutation of FUS/TL; the latter may lead to fulminant, childhood-onset motor neuron disease.\textsuperscript{28}

\section*{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.\textsuperscript{34}

\section*{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,\textsuperscript{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
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), and the proteins optineurin (OPTN), TANK-binding kinase 1 (TBK1), and sequestosome 1 (SQSTM1/p62) (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, 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. Mislocalization and post-translational modification of TDP-43 are observed in many neurode-

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*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.
generative diseases. FUS-TLS encodes another RNA-binding protein, homologous to TDP-43, which in mutant form also causes ALS. 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. This auto-aggregation is facilitated in stress granules, which are nonmembrane-bound structures formed under cell stress that contain RNA complexes stalled in translation. 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. 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. A reduction in the total levels of the normal C9ORF72 protein may also contribute to neurotoxicity. Transgenic mouse models of C9orf72 recapitulate the molecular features of C9ORF72 ALS in humans but, with one exception, 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), PFN1, and tubulin 4A (TUBA4A) (Fig. 4C). 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%. 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. 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, and TBK1 mutations). 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.

Combinations of such gene variants further increase the risk of ALS and may be another cause of apparently sporadic ALS. Recent genomewide association studies have shown that rare genetic variation is disproportionately frequent in sporadic ALS. 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.

Heritability studies also show that a substantial fraction of cases of sporadic ALS cannot be attributed to genetic or biologic factors; these...
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-
ing has been shown to shorten survival in a population study, so a case–control study selecting patients 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, 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. The exposure with the strongest support is military service. In addition, smoking has been implicated as a dose-dependent risk factor for ALS. Exposure to heavy metals may be important; blood lead levels and cerebrospinal fluid manganese levels are higher in patients with ALS than in controls. 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 were followed by the identification of a possible candidate, human endogenous retrovirus K.

There is increasing evidence that trauma precedes some individual cases of ALS. 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. 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.

Therapeutics and Beyond
No therapy offers a substantial clinical benefit for patients with ALS. The drugs riluzole 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. 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...
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 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”. 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 levsimendan 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.

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