

*Medical Progress***AMYOTROPHIC LATERAL SCLEROSIS**LEWIS P. ROWLAND, M.D.,  
AND NEIL A. SHNEIDER, M.D., PH.D.

**C**HARCOT described amyotrophic lateral sclerosis (ALS) in 1874. Despite progress, this creeping paralysis, known colloquially as Lou Gehrig's disease, is still not visibly affected by available therapies. However, advances in genetics have accelerated the pace of ALS research in the past decade, promising more effective treatment.

**DEFINITION OF THE DISEASE**

ALS has two meanings. In one sense, it refers to several adult-onset conditions characterized by progressive degeneration of motor neurons (Fig. 1). In the United Kingdom, the term motor neuron disease is used for these disorders. In the second sense, ALS refers to one specific form of motor neuron disease in which there are both upper and lower motor neuron signs.

"Amyotrophic" refers to the muscle atrophy, weakness, and fasciculation that signify disease of the lower motor neurons. "Lateral sclerosis" refers to the hardness to palpation of the lateral columns of the spinal cord in autopsy specimens, where gliosis follows degeneration of the corticospinal tracts. The clinical results are upper motor neuron signs: overactive tendon reflexes, Hoffmann signs, clonus, and Babinski signs.

If lower motor neuron signs alone are evident, the condition is called progressive spinal muscular atrophy. In primary lateral sclerosis, only upper motor neuron signs are seen. These syndromes are considered variants of ALS because, at autopsy, there are likely to be abnormalities in both upper and lower motor neurons. Together, the syndromes account for only 10 percent of all cases of adult-onset motor neuron disease. In patients with typical ALS, the symptoms are primarily those of weakness, which may start in the hands or legs or be manifested by slurred speech and dysphagia. On examination there are almost always lower motor neuron signs together with upper motor neuron signs. The disease is progressive; the mean duration of survival is three to five years.

**DIAGNOSIS**

The clinical diagnosis of ALS is probably correct in more than 95 percent of cases.<sup>1</sup> However, because

there is no specific diagnostic test, it is sometimes difficult to separate ALS from other motor neuron diseases (especially Kennedy's disease, or X-linked spinobulbar muscular atrophy), cervical spondylotic myelopathy, or myasthenia gravis. Formal criteria are used for clinical trials but may be too restrictive; some patients die of ALS without qualifying for a therapeutic trial.<sup>2</sup>

Perhaps the most important disorder in the differential diagnosis is multifocal motor neuropathy, which is dominated by lower motor neuron signs and characterized by multiple motor-conduction blocks on electrical testing. It accounts for 2 percent of patients seen in ALS centers. Antibodies against the GM<sub>1</sub> ganglioside are found in 22 to 84 percent of patients with multifocal motor neuropathy.<sup>3,4</sup> Unlike ALS, multifocal motor neuropathy responds to treatment with cyclophosphamide<sup>3</sup> or intravenous immune globulin.<sup>5</sup> Intravenous immune globulin therapy may result in improvement in patients with the clinical syndrome of multifocal motor neuropathy who have slowing of conduction<sup>6</sup> or no conduction abnormality at all.<sup>7</sup> Although multifocal motor neuropathy is a peripheral neuropathy, many patients have active tendon reflexes in limbs with atrophic and fasciculating muscles, an incongruous pattern consistent with the diagnosis of ALS. In lower motor neuron syndromes, tendon reflexes should disappear, so the preservation of these responses can be viewed as evidence of upper motor neuron involvement. Reports of autopsy findings in four patients with multifocal motor neuropathy described the loss of motor neurons; some showed intraneuronal inclusions called Bunina bodies, which may be pathognomonic of motor neuron disease.<sup>1,8</sup>

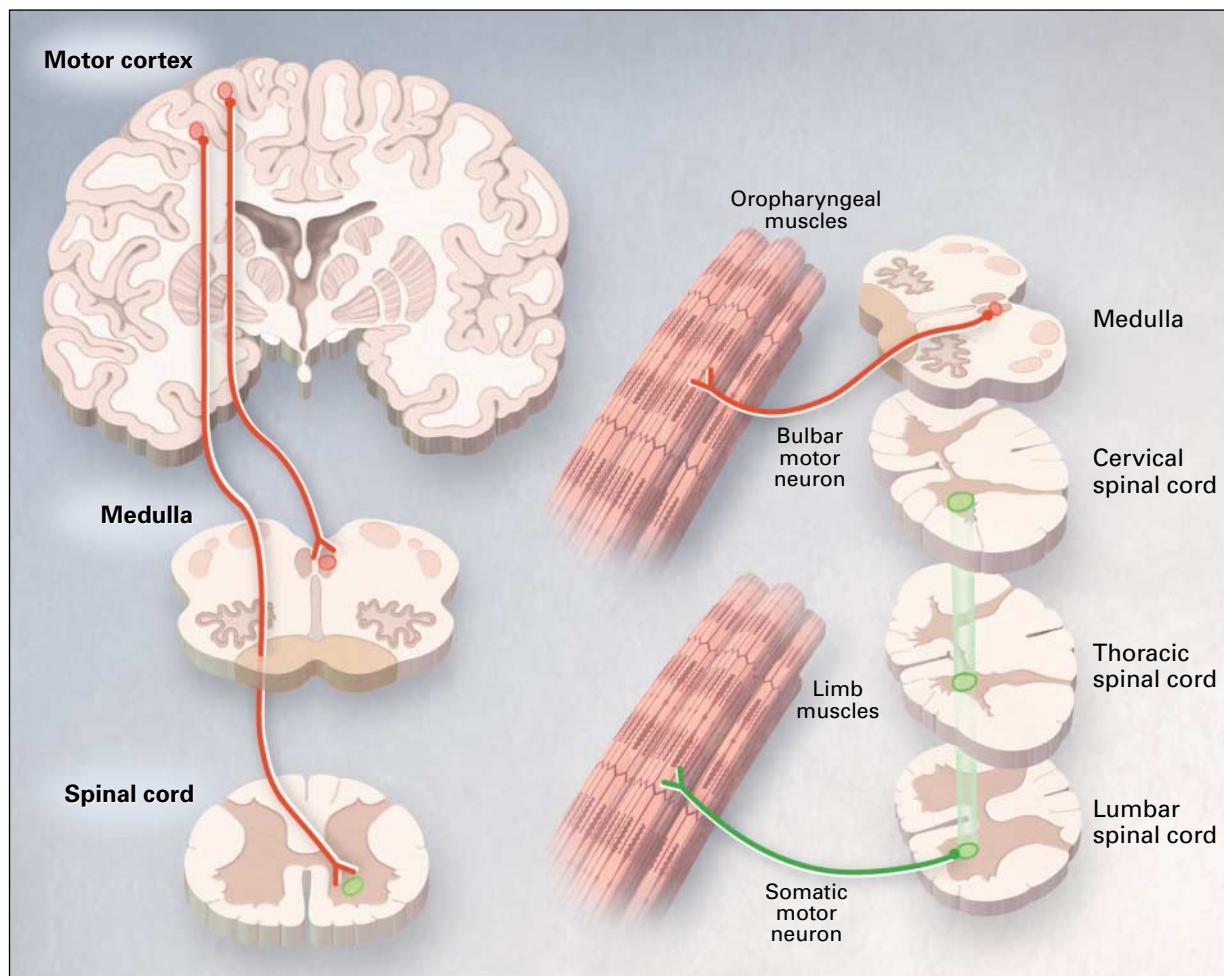
Electromyographic demonstration of denervation in at least three limbs confirms the findings of lower motor neuron abnormalities. The use of electromyography to count the number of surviving motor neurons may become an objective measure of the efficacy of drug therapy.<sup>9,10</sup>

Documenting the involvement of upper motor neurons in patients with ALS could help differentiate ALS from multifocal motor neuropathy and may represent another objective measure of the response to treatment. Two methods are being used. Magnetic resonance spectroscopy<sup>11,12</sup> measures the number of surviving neurons in the motor cortex, and magnetic stimulation of the motor cortex<sup>13</sup> assesses conduction in the corticospinal tracts. The sensitivity and specificity of the two approaches seem to be equal and need to be improved. Magnetic resonance imaging may show high signal intensity in the corticospinal tracts.<sup>11</sup>

**PROPOSED UNDERLYING CAUSES****Genetic Causes****Familial Motor Neuron Diseases**

Heritable diseases are the only motor neuron diseases whose causes are known (Table 1).<sup>14</sup> Five to 10

From the Eleanor and Lou Gehrig MDA/ALS Center, Neurological Institute, Columbia–Presbyterian Medical Center, New York–Presbyterian Hospital, and Columbia University College of Physicians and Surgeons — all in New York. Address reprint requests to Dr. Rowland at the Neurological Institute, Columbia–Presbyterian Medical Center, 701 W. 168th St., New York, NY 10032, or at lpr1@columbia.edu.



**Figure 1.** Motor Neurons Selectively Affected in ALS.

Degeneration of motor neurons in the motor cortex leads to clinically apparent signs of upper motor neuron abnormalities: overactive tendon reflexes, Hoffmann signs, Babinski signs, and clonus. Degeneration of motor neurons in the brain stem and spinal cord causes muscle atrophy, weakness, and fasciculation.

percent of cases of ALS are familial; the others are believed to be sporadic. In 1993, Rosen et al.<sup>15</sup> described mutations in the gene encoding superoxide dismutase 1 (SOD1) that account for 20 percent of cases of familial ALS. The remaining 80 percent are caused by mutations in other genes. Five percent of people with apparently sporadic ALS also have SOD1 mutations. More than 90 SOD1 mutations involve 40 of the 153 amino acid residues. All SOD1 mutations are dominant, except for the substitution of alanine for aspartate at position 90 (D90A), which can be either recessive<sup>16</sup> or dominant.<sup>17</sup> The substitution of valine for alanine at position 4 (A4V) is the most common SOD1 mutation.

Different SOD1 mutations cause distinct syndromes<sup>18,19</sup> that differ with respect to penetrance (pen-

etrance is usually 100 percent but is sometimes less), SOD1 activity of erythrocytes (activity is usually normal but is sometimes depressed), age at onset (onset is usually after the age of 40 but sometimes occurs at a younger age), survival (survival ranges from 1 to 20 years), and clinical manifestations (the initial symptoms may be spinal or bulbar in nature). The histopathological findings also vary. In patients with the A4V mutation in SOD1, the corticospinal tracts are largely spared.<sup>18</sup> Neuronal inclusions are not always present; for example, they may be present in some family members and absent in others.

Another autosomal dominant form of ALS progresses slowly and begins before the age of 25 years<sup>20</sup>; the gene has been mapped to chromosome 9q34.<sup>21</sup> The gene for ALS with frontotemporal dementia has

**TABLE 1.** CLASSIFICATION OF HEREDITARY MOTOR NEURON DISEASES.\*

DISEASE†	MAIN MODE OF INHERITANCE	CLINICAL FEATURES‡		LINKAGE	PROTEIN AFFECTED	FEATURES
		UMN SIGNS	LMN SIGNS			
<b>Spinal muscular atrophy</b>						
Type 1 (Werdnig–Hoffmann disease)	Autosomal recessive (rarely X-linked)	None	+++	5q11.2–13.3	Survival motor neuron	Onset between birth and 6 mo of age; death before the age of 2 yr
Type 2 (intermediate)	Autosomal recessive	None	+++	5q11.2–13.3	Survival motor neuron	Onset before 1 yr of age; children never able to stand; death after the age of 2 yr
Type 3 (Wohlfart–Kugelberg–Welander disease)	Autosomal recessive or autosomal dominant	None	+++	5q11.2–13.3	Survival motor neuron	Onset in childhood or adolescence; course varies but is often mild
Type 4 (adult onset)	Autosomal recessive or autosomal dominant	None	+++	5q11.2–13.3	Survival motor neuron	Onset after the age of 25 yr; mild
Distal (neuronal form of Charcot–Marie–Tooth disease)	Autosomal recessive or autosomal dominant	None	+++	8p21	Neurofilament light chain	Onset in adolescence; distal weakness; normal nerve conduction velocity
<b>Kennedy’s disease (X-linked spinobulbar muscular atrophy)</b>	X-linked recessive	None	++	Xq21–22	Androgen receptor (increased numbers of CAG repeats in gene)	Onset in adolescence or later; pure lower motor neuron syndrome; fasciculation prominent; often accompanied by gynecomastia; slow rate of progression
<b>Familial ALS</b>						
ALS	Autosomal dominant	++	+++	21q22.1	Superoxide dismutase	ALS alone
ALS with frontotemporal dementia	Autosomal dominant	++	+++	9q21–22	Unknown	ALS; dementia
ALS with frontotemporal dementia and parkinsonism	Autosomal dominant	++	+	17q21	Tau	Dementia; parkinsonism; amyotrophy
ALS	X-linked	++	+++	Xp11–Xq12	Unknown	ALS alone
Juvenile type 1	Autosomal recessive	+	++	15q15–22	Unknown	ALS alone
Juvenile type 2	Autosomal recessive	+	+	Unknown	Unknown	ALS alone
Juvenile type 3	Autosomal recessive	+++	+	2q33	Unknown	ALS alone
Juvenile	Autosomal dominant	++	++ (no bulbar)	9q34	Unknown	ALS alone; onset before the age of the 25 yr
<b>Sporadic ALS</b>	None	++	+++	None	Unknown	ALS alone
<b>Hereditary spastic paraplegia§</b>	Autosomal dominant, autosomal recessive, or X-linked	+++	None	>15 Loci	Paraplegin, cellular adhesion molecule, proteolipid protein, spastin, others unknown	Spastic paraparesis

\*Data have been modified from Cole and Siddique.<sup>14</sup>

†Alternative terms for disease are given in parentheses.

‡UMN denotes upper motor neuron, and LMN lower motor neuron. The frequency and prominence of each sign are indicated by the number of plus signs: low (one plus sign), intermediate (two plus signs), and high (three plus signs).

§Some forms of hereditary spastic paraplegia may be subcortical in origin, as occurs in demyelinating disease (e.g., proteolipid protein is responsible).

been mapped to 9q21–22.<sup>22</sup> Autosomal recessive juvenile-onset ALS has been linked to chromosomes 2q33<sup>23</sup> and 15q15–22.<sup>24</sup>

**Genetic Susceptibility**

ALS and other neurodegenerative disorders sometimes appear in the same family. Majoor-Krakauer et al.<sup>25</sup> found dementia significantly more often in the first-degree relatives of patients with ALS than in rel-

atives of control subjects. They found a trend toward an association between ALS and parkinsonism. Cruz et al.<sup>26</sup> found no such associations, but some persons and families have both ALS and parkinsonism.<sup>27,28</sup> The occurrence of the two disorders together could be due to chance or to multisystem diseases. Amyotrophy is found with dementia and parkinsonism in patients with the chromosome 17–linked disease with mutations in the gene for tau, an intermediate filament im-

portant in the cytostructure of neurons.<sup>29</sup> ALS and dementia also occur together in the disease whose chromosomal location was mapped to 9q21–22.<sup>22</sup>

Age and a family history of ALS are the only established risk factors for ALS. Apparent clusters of disease are attributed to chance, but a founder effect may be responsible in some areas with clusters of autosomal dominant familial ALS.<sup>30</sup>

### Environmental Causes

#### *Epidemiologic Features*

The incidence and prevalence of ALS vary little worldwide, with notable pockets of higher prevalence, especially in Guam. During World War II, neuropathologist Harry Zimmerman noted an unusual frequency of ALS, parkinsonism, and dementia in Guam. Epidemiologic studies indicated that the prevalence of ALS in Guam was 50 times the prevalence anywhere else.<sup>31</sup> Both the parkinsonism–dementia–ALS complex and ALS alone remain prevalent in Guam.

The cause of Guamanian ALS with parkinsonism and dementia is unknown. Heredity was discounted because the spouses of many patients were also affected, and no environmental cause or virus was found.<sup>32</sup>

#### *Exposure to Heavy Metals*

Many neurologists order tests for the measurement of mercury, lead, and arsenic in blood and urine. However, there is doubt that mercury or arsenic has ever caused ALS. Lead intoxication once caused a syndrome involving both upper and lower motor neurons, but the syndrome disappeared once occupational exposure to lead began to be monitored. There has not been a convincing report of lead-induced motor neuron disease for 25 years.

### Viral Infection and Prion Disease as Causes

Persistent viral infection might cause sporadic ALS. Berger et al. detected enterovirus RNA in the spinal cords of patients with ALS,<sup>33</sup> but that observation was not confirmed,<sup>34</sup> and the role of enteroviruses, including poliovirus, has not been established.<sup>35</sup> Motor neuron disease has also been reported in a small number of patients infected with the human immunodeficiency virus (HIV) or human T-cell lymphotropic virus type I, but the existence of these few cases does not prove that retroviral infection causes motor neuron disease. In exceptional cases, anti-HIV therapy has reversed the motor neuron syndrome. Lyme disease in rare cases causes a syndrome with both upper and lower motor neuron signs, but it does not cause typical ALS.<sup>36</sup>

There was once thought to be an amyotrophic form of Creutzfeldt–Jakob disease. In 1983, however, Salazar et al.<sup>37</sup> reported that the injection of brain tissue from 33 patients who had ALS with dementia did not transmit the disease to monkeys, except in the

case of 2 patients with “atypical” features. Prion disease seemed an unlikely cause of ALS. Later, however, it was recognized that 3 of the 33 cases were transmitted, and the atypical features were compatible with the features of amyotrophy in patients with Creutzfeldt–Jakob disease.<sup>38</sup> In 50 cases of proven prion disease, lower motor neuron signs were recorded.<sup>38</sup>

### Alternative Theories

Autoimmunity may have a role in pathogenesis.<sup>39</sup> Activated microglia and T cells have been found in the spinal cords of patients with ALS who have IgG antibodies against motor neurons.<sup>40</sup> In patients with sporadic ALS, antibodies against voltage-gated calcium channels may interfere with the regulation of intracellular calcium, leading to the degeneration of motor neurons.<sup>40</sup> This process has been verified by electron-microscopical findings.<sup>41</sup>

However, immunotherapy has not been effective in patients with ALS. Corticosteroids, plasmapheresis, intravenous immune globulin, cyclophosphamide, and whole-body radiation have all failed. The theory of an autoimmune cause of ALS is controversial.<sup>42</sup>

Paraneoplastic motor neuron disease could be an autoimmune disorder. Epidemiologic studies have not shown an unexpectedly high number of malignant tumors among patients with ALS, but the neurologic syndrome in these patients sometimes abates after the removal of a tumor of lung or kidney. Some patients with cancer and ALS were found to have antineuronal antibodies.<sup>43–46</sup>

The incidence of lymphoproliferative diseases among patients with motor neuron diseases may be higher than expected.<sup>47–49</sup> Of the 65 reported cases of ALS with lymphoproliferative disease, half involved both upper and lower motor neuron signs. Eighty percent had Hodgkin’s or non-Hodgkin’s lymphoma, and the other 20 percent had myeloma or macroglobulinemia. Among these patients, few had a neurologic response to immunotherapy and most died of the neuronal disease. Many patients with ALS have a monoclonal gammopathy whether or not they have a lymphoproliferative disease, but the nature of the association is not known. Both motor neuron disease and lymphoproliferative disease could arise from a persistent viral infection, as is the case in wild mice with a spontaneous retroviral infection that causes both leukemia and motor neuron disease.

### HISTOPATHOLOGICAL FEATURES

The pathological hallmarks of ALS are the degeneration and loss of motor neurons with astrocytic gliosis. Intraneuronal inclusions are seen in degenerating neurons and glia<sup>50,51</sup> (Table 2). The finding of similar inclusion bodies in patients with ALS and in those with ALS dementia led Ince et al.<sup>52</sup> to posit the existence of a spectrum of disease ranging from pure frontotemporal dementia to pure motor neu-

TABLE 2. INTRANEURONAL INCLUSIONS OF ALS.

INCLUSION	FEATURES	COMMENT
Bunina bodies	Eosinophilic Hyaline Intracytoplasmic Positive for cystatin (an inhibitor of cysteine protease)	Found in about 70 percent of patients at autopsy Rarely seen in other conditions, so both the sensitivity and specificity of this finding are high
Ubiquitinated inclusions*	Do not react with antibodies against neurofilament or tau, unlike the ubiquitinated inclusions of other neurodegenerative diseases	Found in skein-like inclusions in patients with ALS Found in several other neurodegenerative diseases including Alzheimer's disease (neurofibrillary tangles) and Parkinson's disease (Lewy bodies)
Lewy-like bodies	Resemble Lewy bodies but may contain neurofilaments	May be related to skein-like inclusions, but are less common
Conglomerate hyaline inclusions	Stain intensely for phosphorylated and nonphosphorylated neurofilaments Weakly positive for ubiquitin	In some patients with familial ALS, inclusions contain immunoreactive superoxide dismutase 1 or neurofilaments
Advanced glycated end products	Insoluble proteins in neuronal hyaline inclusions Contain ubiquitin, phosphorylated neurofilament, and superoxide dismutase 1 Deposited by a process of glycation and oxidation	Found in patients with familial ALS with the A4V mutation in the gene for superoxide dismutase 1

\*Ubiquitin is thought to form covalent bonds with other proteins in order to mark them for degradation by an ATP-dependent, nonlysosomal, proteolytic system.

ron disease and syndromes of combined ALS and dementia.

Mitochondrial abnormalities have been found in patients with ALS and transgenic mice with mutant SOD1.<sup>53,54</sup> Only two cases of motor neuron disease have been associated with mutations in mitochondrial DNA.<sup>55,56</sup> Some patients also have fragmentation of the Golgi apparatus.<sup>57</sup>

### PATHOGENESIS

Although the precise molecular pathways that cause the death of motor neurons in ALS remain unknown,<sup>58,59</sup> possible primary mechanisms include the toxic effects of mutant SOD1, including abnormal protein aggregation; the disorganization of intermediate filaments; and glutamate-mediated excitotoxicity and other abnormalities of intracellular calcium regulation in a process that may involve mitochondrial abnormalities and apoptosis (Fig. 2).

#### SOD1-Induced Toxicity

Sporadic and familial ALS are clinically and pathologically similar, suggesting a common pathogenesis. Although only 2 percent of patients with ALS have a mutation in *SOD1*, the discovery of these mutations<sup>15</sup> was a landmark in ALS research because it provided the first molecular insights into the pathogenesis of the disease.

SOD1, an enzyme that requires copper, catalyzes the conversion of toxic superoxide radicals to hydrogen peroxide and oxygen. A copper atom at the active site mediates catalysis. SOD1 also has pro-oxidant activities, including peroxidation, the generation of hydroxyl radicals, and the nitration of tyrosine (Fig. 3).

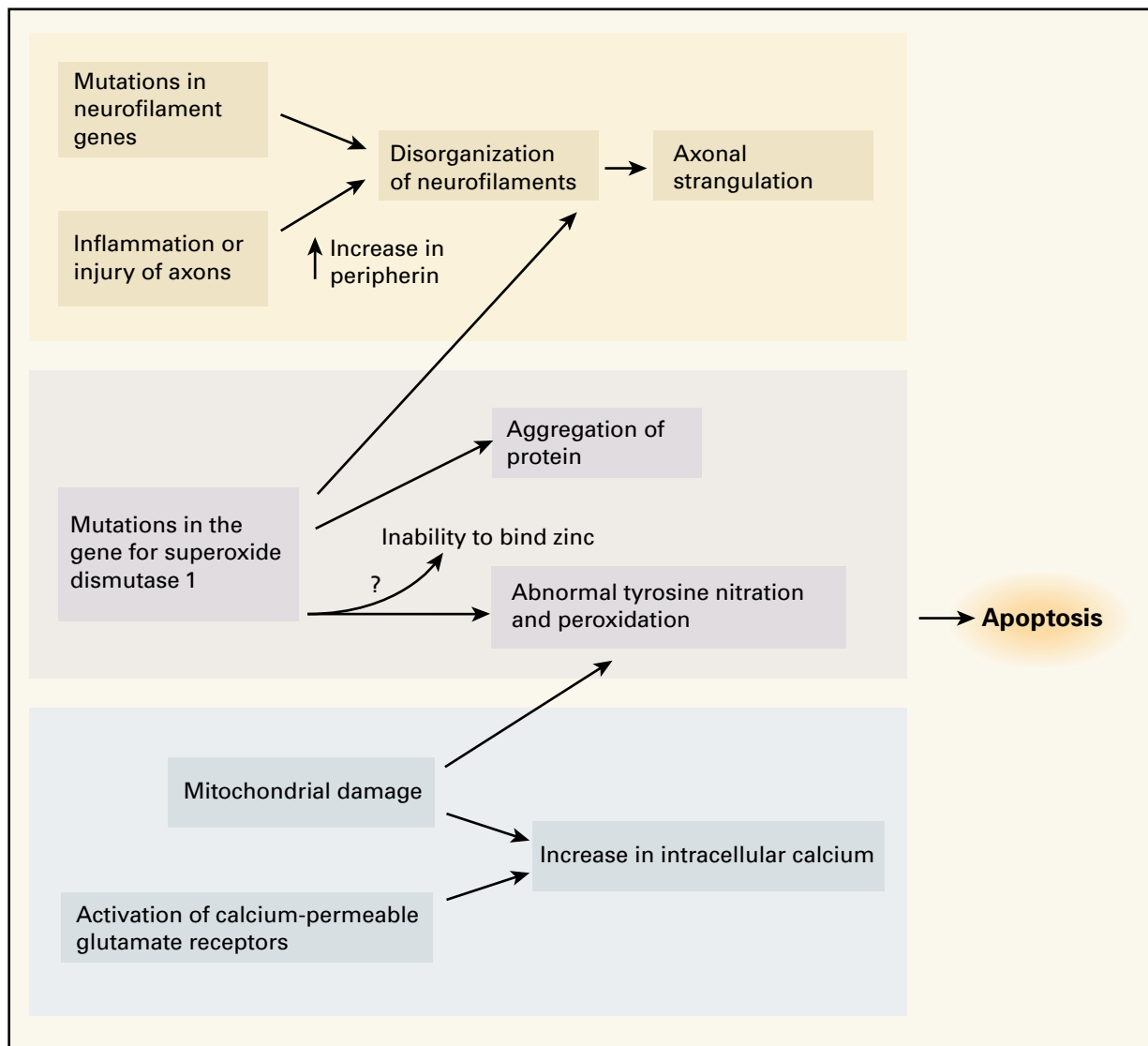
Mutations in *SOD1* that impair the antioxidant

functions of the enzyme could lead to toxic accumulation of superoxide.<sup>60,61</sup> This loss-of-function hypothesis was disproved, because the overexpression of mutant SOD1 (in which alanine had been substituted for glycine at position 93 of *SOD1* [G93A]) in mice caused motor neuron disease despite the presence of elevated SOD1 activity.<sup>62</sup> Moreover, the total elimination of SOD1 did not cause motor neuron disease in mice in which *SOD1* has been inactivated, or "knocked out."<sup>63</sup> Therefore, *SOD1* mutations must cause disease by a toxic gain of function, not by the loss of the scavenging activity of SOD1.

#### Peroxynitrite and Zinc

According to one gain-of-function theory, a mutation in *SOD1* alters the enzyme in a way that enhances its reactivity with abnormal substrates (Fig. 3). For example, abnormal tyrosine nitration could damage proteins if the radical peroxynitrite is used as a substrate of SOD1.<sup>64</sup> Spinal cord levels of free nitrotyrosine are elevated in patients with sporadic ALS and in those with familial ALS,<sup>65</sup> as well as in *SOD1*-knockout mice,<sup>66</sup> but specific targets of nitration have not been identified.

Mutations in *SOD1* may cause oxidative damage by impairing the ability of the enzyme to bind zinc.<sup>67</sup> Deprived of zinc, both mutant and wild-type SOD1 are less efficient superoxide scavengers, and the rate of tyrosine nitration increases.<sup>68</sup> Mutations in *SOD1* decrease the enzyme's affinity for zinc,<sup>68</sup> so that the mutant protein is more likely to assume a toxic, zinc-deficient state. It has also been theorized that in patients with sporadic ALS, normal SOD1 might also somehow be stripped of zinc to become toxic.



**Figure 2.** Mechanisms That May Contribute to the Degeneration of Motor Neurons in ALS.

#### **Copper and SOD1 Aggregates**

Zinc-deficient SOD1 still requires copper at the active site even though its activity is abnormal. Two chelators remove copper from zinc-deficient SOD1 but not from normal SOD1 (replete with both copper and zinc).<sup>67</sup> Both chelators protected cultured motor neurons from zinc-deficient SOD1<sup>67</sup> and might be beneficial in treating human ALS.

Despite this finding, it is uncertain whether SOD1-induced toxicity requires any enzymatic activity — normal or abnormal. A copper chaperone protein for SOD1 incorporates copper ions into both wild-type and mutant SOD1.<sup>69</sup> In mice, targeted disruption of the gene for this chaperone protein markedly reduced but did not eliminate SOD1 activity in the central

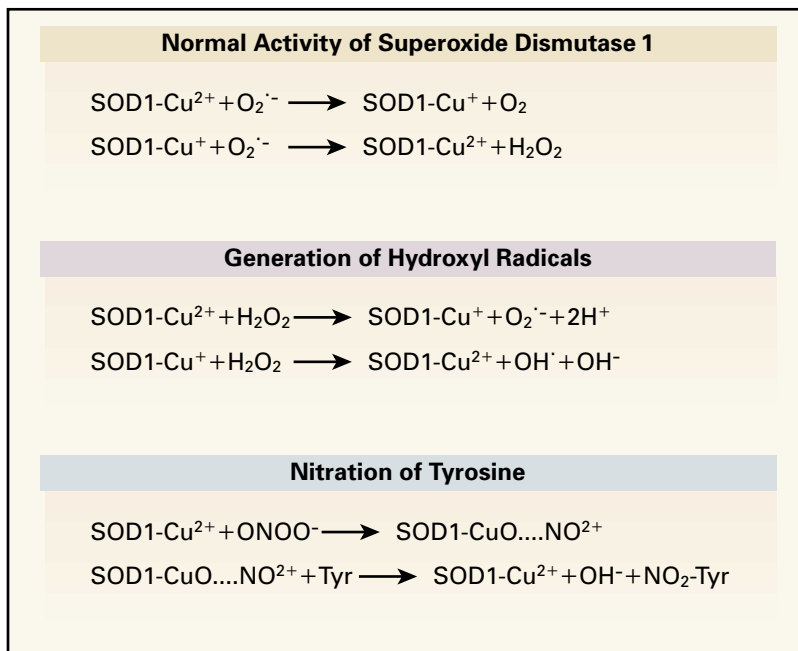
nervous system.<sup>70</sup> If copper loading could be eliminated in a mouse with a mutation in *SOD1*, it would be possible to determine whether copper-mediated catalysis is required for the toxic effect.

SOD1-mediated oxidative abnormalities may not be a primary cause of toxicity. Instead, the proposed toxic gain-of-function mechanism may involve misfolding of mutant SOD1 to form abnormal protein aggregates,<sup>71,72</sup> as occurs in age-related neurodegenerative disorders.

#### **Disorganization of Intermediate Filaments**

##### **Neurofilaments**

Possible targets of SOD1-induced toxicity include the neurofilament proteins, which are composed of



**Figure 3.** Copper-Mediated Oxidative Reactions Catalyzed by Superoxide Dismutase 1.

Superoxide dismutase 1 (SOD1) normally catalyzes the conversion of toxic superoxide anions ( $\text{O}_2^{\cdot-}$ ) to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (top). Mutations in the gene for superoxide dismutase 1 may reverse this reaction, leading to the production of toxic hydroxyl radicals ( $\text{OH}^{\cdot}$ ) (middle), or promote the use of other abnormal substrates such as peroxynitrite ( $\text{ONOO}^-$ ), ultimately leading to the aberrant nitration of tyrosine residues (Tyr) in proteins (bottom).

heavy, medium, and light subunits. They have a role in axonal transport and in determining the shape of cells and the caliber of axons. Large-caliber, neurofilament-rich motor axons are preferentially affected in human ALS, and the level of neurofilaments may be important in selective neuronal vulnerability.

In both patients with sporadic ALS and those with familial ALS,<sup>73,74</sup> as well as in *SOD1*-knockout mice,<sup>75,76</sup> neurofilaments accumulate in the cells and proximal axons of motor neurons. Abnormalities in neurofilaments could be either causal or a byproduct of neuronal degeneration.<sup>77</sup>

The direct involvement of neurofilaments in pathogenesis was suggested by the finding that overexpression of mutant<sup>78</sup> or wild-type<sup>79,80</sup> subunits in mice caused the dysfunction of motor neurons and the degeneration of axons and resulted in neurofilament swellings that were similar to those seen in patients with ALS. Also, mutations in the gene for the heavy subunit of neurofilaments are found in patients with sporadic ALS and in those with familial ALS.<sup>81,82</sup> A mutation in the gene for the light subunit of neurofilaments was found in another motor neuron disorder, the neuronal form of Charcot-Marie-Tooth disease.<sup>83</sup>

The way in which the aberrant expression of neurofilaments causes the degeneration of motor neurons is unclear. Disorganized neurofilaments could impede the axonal transport of molecules necessary for the maintenance of axons (referred to as “axonal strangulation”) (Fig. 2).<sup>84,85</sup> Such abnormalities in neurofilaments may result from the toxic effects of mutant SOD1. In mice with a mutation in *SOD1*, elimination of the expression of the light subunit of neurofilaments<sup>86</sup> or overexpression of the heavy subunit of neurofilaments<sup>87</sup> ameliorated the motor neuron disease. Axonal neurofilaments may be targets of the toxic effects of mutant SOD1, which could explain why reducing the number of axonal neurofilaments is protective. Alternatively, the accumulation of neurofilaments in motor neuron cells could protect against SOD1-mediated injury by buffering calcium<sup>88</sup> or diminishing zinc binding.

#### Peripherin

Peripherin — another intermediate filament — is found with neurofilaments in the neuronal inclusions of patients with sporadic ALS<sup>89</sup> and mice with *SOD1* mutations.<sup>90</sup> Peripherin is normally expressed in motor neurons,<sup>91,92</sup> but levels of peripherin in-

crease in response to cellular injury<sup>91</sup> or inflammatory cytokines.<sup>93</sup> Overexpression of peripherin in mice induced selective degeneration of motor axons.<sup>94</sup> The levels of messenger RNA (mRNA) of the light subunit of neurofilaments are abnormally low in the neurons of patients with sporadic ALS.<sup>95</sup> In mice that lack these light subunits and also overexpress peripherin, the selective death of motor neurons is a prominent characteristic.

Therefore, increased expression of peripherin after neuronal injury or inflammation could cause motor neuron disease through an interaction with the medium and heavy subunits of neurofilaments in the absence of the light subunits,<sup>96</sup> leading to the formation of toxic aggregates. This could explain why the overexpression of peripherin kills only motor neurons, which contain high levels of neurofilaments, and not sensory neurons,<sup>94</sup> which do not express neurofilaments.

### Calcium Homeostasis and Excitotoxicity

#### Calcium-Binding Proteins

There is much evidence to indicate that ALS involves a derangement of intracellular free calcium. Abnormal calcium homeostasis activates a train of events that ultimately triggers cell death. In patients with ALS and in mice with mutant SOD1,<sup>97</sup> the resistance of particular motor neurons (e.g., oculomotor neurons) may be related to the presence of calcium-binding proteins that protect against the toxic effects of high intracellular calcium levels.<sup>98,99</sup>

#### Glutamate Receptors and Transporters

The mechanism of excitotoxic injury of neurons involves excessive entry of extracellular calcium through the inappropriate activation of glutamate receptors. Glutamate, the chief excitatory neurotransmitter in the central nervous system, acts through two classes of receptors: the G protein-coupled receptor, which, when activated, leads to the release of intracellular calcium stores, and the glutamate-gated ion channels, which are distinguished by their sensitivity (or insensitivity) to *N*-methyl-D-aspartic acid (NMDA).

The NMDA-receptor channel is calcium-permeable, whereas the permeability of the non-NMDA-receptor channel (activated by the selective agonists kainate and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid [AMPA]) varies with the subunit composition of the receptor. If a particular subunit (named GluR2) is present, the channel is impermeable to calcium. In contrast, AMPA receptors that lack GluR2 are calcium-permeable. This activity of the GluR2 subunit depends on post-transcriptional editing of GluR2 mRNA.<sup>100</sup> The selective vulnerability of motor neurons to AMPA<sup>101</sup> could be explained either by the fact that the expression of GluR2 in motor neurons is normally lower than in other neurons<sup>102</sup> or by an impairment in the editing of

GluR2 mRNA in patients with ALS.<sup>103</sup> Either mechanism would lead to the expression of calcium-permeable AMPA receptors.

The possibility of glutamate excitotoxicity in patients with ALS<sup>104,105</sup> was suggested by the finding of increased glutamate levels in cerebrospinal fluid in patients with sporadic ALS.<sup>106,107</sup> High levels of glutamate could be excitotoxic, increasing levels of free calcium through the direct activation of calcium-permeable receptors or voltage-gated calcium channels.

The increased levels of glutamate in cerebrospinal fluid could also result from impaired glutamate transport in the central nervous system. The synaptic activity of glutamate is normally terminated by reuptake of the neurotransmitter by excitatory amino acid transporters (EAATs), predominantly<sup>108</sup> the EAAT1 and EAAT2 proteins on perisynaptic astrocytes. Rothstein<sup>109</sup> proposed that the selective loss of EAAT2 in patients with sporadic ALS impairs glutamate transport. This loss of EAAT2 was attributed to aberrant splicing of EAAT2 mRNA in affected regions of the central nervous system.<sup>110</sup> The presence of disease-specific and region-specific errors in the processing of EAAT2 mRNA, however, has not been confirmed.<sup>111-113</sup>

In patients with familial ALS, mutant SOD1 could lead to excitotoxic neuronal injury by catalyzing the inactivation of EAAT2, as it does in the presence of hydrogen peroxide.<sup>114</sup> This process would represent another link between familial and sporadic ALS.

Mutant SOD1 may also affect intracellular calcium levels through a direct toxic effect on mitochondria, which are essential for calcium homeostasis.<sup>115,116</sup> The high metabolic load of motor neurons and the consequent dependence of these cells on oxidative phosphorylation may make them particularly vulnerable to the loss of mitochondrial function.

### Apoptosis

The many possible triggers of ALS could perturb diverse cellular functions essential for the survival of motor neurons. In SOD1-mediated ALS, motor neurons most likely die as a result of apoptosis,<sup>117</sup> although this point is disputed.<sup>118</sup> Apoptosis involves the activation of the caspase proteases<sup>119</sup> in response to signals integrated by Bcl-2 proteins.<sup>120</sup> In mice with the G93A mutation in *SOD1*, the expression of anti-apoptotic Bcl-2 delayed the onset of motor neuron disease and prolonged life.<sup>121</sup> An inhibitor of the caspase, interleukin-1 $\beta$ -converting enzyme, also slowed progression and extended survival,<sup>122</sup> as did the intracerebroventricular administration of zVAD-fmk, a broad caspase inhibitor.<sup>123</sup> Although apoptosis is a late event in the degeneration of motor neurons, inhibition of programmed cell death might ameliorate ALS.

Multiple theories have been proposed to explain the molecular pathogenesis of ALS. It is likely that more than one of these mechanisms contributes to



human ALS. How these pathways interact remains to be explained.

## THERAPY

### Pharmacotherapy

Riluzole, a glutamate antagonist, is the only drug approved by the Food and Drug Administration for the treatment of ALS (Table 3). In two therapeutic trials, riluzole prolonged survival by three to six months.<sup>124,125</sup> In one of these trials,<sup>124</sup> treatment slightly slowed the decline in the strength of limb muscle; there was no benefit with respect to many measures of function in either trial. In one retrospective analysis,<sup>126</sup> patients who received riluzole remained in a milder stage of disease longer than did controls. For patients, the effects are invisible. The efficacy of riluzole has been taken as evidence in support of the excitotoxic-glutamate theory of the pathogenesis of ALS. But other glutamate antagonists, including branched-chain amino acids, lamotrigine, and dextromethorphan, had no beneficial effects in clinical trials.<sup>127,128</sup>

When tested in transgenic mice with mutant SOD1, gabapentin, like riluzole, extended survival but did not significantly affect the onset of clinical disease.<sup>129</sup> In contrast, vitamin E delayed the onset and the progression of the disease but failed to extend survival. Despite the moderate benefits of these agents in mice, gabapentin and vitamin E were of no benefit in trials of patients with ALS.<sup>130,131</sup>

More than 60 years ago, Wechsler touted the benefits of vitamin E in a series of patients with ALS.<sup>132</sup> Although Wechsler reported an improvement in the condition of Patient 4, identified on the basis of his initials and age as Lou Gehrig himself, Gehrig nevertheless died within a year. Other treatments have also failed in clinical trials (Table 3). Agents that are currently being evaluated include xaliproden (which may foster the release of neurotrophic factors), creatine,<sup>133</sup> coenzyme Q10, intrathecally administered (by lumbar puncture) brain-derived neurotrophic factor, and orally administered brain-derived neurotrophic factor.<sup>134</sup> Inhibitors of cyclooxygenase-2<sup>135</sup> and caspase inhibitors are being considered, and “high-throughput” drug development is on the horizon.<sup>136</sup> Reliable cell-based or other in vitro assays are needed to expedite the process of identifying potential therapies.

### Mechanical Ventilatory Support

The central problem of treatment is the decision ultimately faced by all patients: whether to elect to undergo a tracheostomy for long-term mechanical ventilation. That choice can be postponed by the use of noninvasive positive-pressure ventilation, which relieves symptoms and prolongs life. Few patients actually agree to the use of mechanical ventilation, because it invokes the prospect of years of total immobility

TABLE 3. THERAPY FOR ALS.

CLASS	DRUG OR PREPARATION
Glutamate antagonists	Riluzole*
	Lamotrigine†
	Dextromethorphan†
	Gabapentin†
Antioxidants	Branched-chain amino acids‡
	Vitamin E†
	Acetylcysteine†
	Selegiline†
	Creatine‡
	Selenium
Neurotrophic factors	Coenzyme Q10‡
	Brain-derived neurotrophic factor†
	Insulin-like growth factor 1†
	Glial-derived neurotrophic factor†
	Xaliproden‡
Immunomodulatory agents or approaches	Thyrotropin-releasing hormone†
	Gangliosides
	Interferon
	Cyclophosphamide†
	Plasmapheresis
	Intravenous immune globulin
	Levamisole†
Transfer factor†	
Antiviral agents	Amantadine†
	Tilorone†
Other agents	Snake venom

\*Riluzole had marginal benefits in clinical trials; it has been approved by the Food and Drug Administration for the treatment of ALS.

†This agent had no beneficial effect in a controlled clinical trial.

‡This agent is currently being evaluated in a clinical trial.

and limited communication and places a heavy burden on their families.

### Treatment for Depression

Because it is widely believed that everyone who is given a diagnosis of ALS becomes depressed, antidepressant drugs are often prescribed, but there have been no trials to evaluate the effects of this practice. In two studies involving 100 patients with ALS, clinical depression was found in only 11 percent.<sup>137,138</sup> Psychological and spiritual considerations are also determinants of the quality of life.<sup>139,140</sup> In addition, health care workers are treating physical symptoms more actively.<sup>141</sup>

### Proposed Treatments

Therapeutic trials have become increasingly well organized, and most have been funded by pharmaceutical companies. The lack of effective treatment has caused many patients and their families to become activists, raising money for research and bypassing traditional granting agencies.<sup>142</sup> This “guerrilla science” approach has led to proposals for gene therapy. Such

approaches must first be attempted in animals to evaluate their safety and efficacy. One approach is to use a viral vector to deliver the gene for EAAT2 into the spinal cord by an intraparenchymal injection in an attempt to lower circulating glutamate levels.<sup>143</sup> The aim of another project is to restore motor function by introducing human stem cells into the spinal cord to replace degenerating motor neurons. Stem-cell therapy for ALS was propelled by four 1999 reports that described how stem cells made their way to the proper location, settled, and replaced dysfunctional cells.<sup>144</sup> In the case of ALS, this approach will be particularly difficult because of the complex pathways involved in motor function. Precise connections have to be restored between motor neurons, target muscle, and descending motor systems. Nevertheless, stem-cell therapy may be of protective value, slowing or preventing further neuronal degeneration.

#### END-OF-LIFE ISSUES

In media stories about assisted suicide, patients with ALS figure prominently. In 1999, the death by euthanasia of a man with ALS was broadcast on national television. Suicide can be viewed as a rational solution by patients who know the toll that ALS takes physically, emotionally, and financially on themselves and their families. The tough question is when: not too soon, when daily functions are still possible, and not too late, when the hands can no longer function. If the hands are paralyzed, someone else must be involved, and the act becomes euthanasia.<sup>145</sup>

Few patients with ALS request assisted suicide, and few opt to receive long-term mechanical ventilation.<sup>146,147</sup> In Oregon, assisted suicide is legal, but few have used that option. In one study,<sup>148</sup> only one patient with ALS expressed interest in committing suicide, although 20 percent of such patients wanted to have a sedative drug available. Among the few who choose to receive long-term ventilation, even fewer request that treatment be terminated. These low numbers may be attributed to the hospice movement, which makes comfort care an alternative to suicide. The use of oral opiates sometimes does not suffice, and terminal sedation<sup>145</sup> then becomes an option; it is legal and ethical to relieve a patient's suffering even if that effort does not prolong life.

#### CONCLUSIONS

ALS is still a fatal disease. Progress in research has been made during the past decade, but it has not yet yielded an effective therapy. Nevertheless, there is reason to hope. Genetic analysis has identified a primary cause of ALS. Mutations in a single gene can initiate a process that leads to the selective degeneration of motor neurons. The clinical and pathological similarities of familial and sporadic ALS suggest a common pathogenesis. The challenge now is to understand how these mutations cause disease and to use

this understanding to develop a treatment, perhaps a cure. The cascade of events that leads to the death of motor neurons is complex. The isolation of genes responsible for other familial forms of ALS should reveal other points in the pathway at which therapeutic intervention may be possible.

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