Neuron 52, 39–59, October 5, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.neuron.2006.09.018

ALS: A Disease of Motor Neurons and Their Nonneuronal Neighbors

Review

Séverine Boillée,[1] Christine Vande Velde,[1] and Don W. Cleveland[1,*]
[1] Ludwig Institute for Cancer Research and Departments of Medicine and Neuroscience University of California, San Diego 9500 Gilman Drive La Jolla, California 92093

Amyotrophic lateral sclerosis is a late-onset progressive neurodegenerative disease affecting motor neurons. The etiology of most ALS cases remains unknown, but 2% of instances are due to mutations in Cu/Zn superoxide dismutase (SOD1). Since sporadic and familial ALS affects the same neurons with similar pathology, it is hoped that therapies effective in mutant SOD1 models will translate to sporadic ALS. Mutant SOD1 induces non-cell-autonomous motor neuron killing by an unknown gain of toxicity. Selective vulnerability of motor neurons likely arises from a combination of several mechanisms, including protein misfolding, mitochondrial dysfunction, oxidative damage, defective axonal transport, excitotoxicity, insufficient growth factor signaling, and inflammation. Damage within motor neurons is enhanced by damage incurred by nonneuronal neighboring cells, via an inflammatory response that accelerates disease progression. These findings validate therapeutic approaches aimed at nonneuronal cells.

Introduction

It has been over 135 years since the great French neurobiologist and clinician Jean-Martin Charcot (Charcot and Joffroy, 1869) first described the progressive, late-onset motor neuron disease Amyotrophic lateral sclerosis (ALS). With premature degeneration and death of upper and lower motor neurons provoking fatal paralysis as its salient clinical features, the name of the disease derives from Charcot's observation in the lateral portions of the spinal cord of a distinct "myelin pallor" representing degeneration and loss of the axons of upper motor neurons as they descend from the brain to connect directly or indirectly onto the lower motor neurons within the spinal cord. Initially known as Charcot's sclerosis and now more familiarly known in the United States as Lou Gehrig's disease, onset of disease is in midlife (usually between age 45 and 60), with a typical disease course of one to five years. Consideration of incidence (frequency of new cases per year) and prevalence (the proportion of affected individuals in the population; 1–2 and 4–6 per 100,000, respectively) understates the impact of ALS, with the lifetime risk at about 1 in 1000 (Mitsumoto et al., 1998; Yoshida et al., 1986).

Most incidences (90%) of ALS are sporadic, that is, without an obvious genetic component. Approximately 10% are inherited in a dominant manner (and referred to as familial ALS). Sporadic and familial ALS produce similar pathological hallmarks, including progressive muscle weakness, atrophy, and spasticity, each of which reflects the degeneration and death of upper and lower motor neurons. Denervation of the respiratory muscles and diaphragm is generally the fatal event. Despite intense effort, very limited therapeutic options have emerged for slowing disease course, although advances have been made in palliative therapy.

We review here what has been learned about new genes, the current models of pathogenesis, and summarize directions now being undertaken for promising new therapies.

Genetics of ALS

Most incidences of ALS are sporadic but ~10% of patients have a familial history. The identified chromosomal loci containing the mutations leading to ALS-like human motor neuron diseases (Table 1) have been defined as ALS1 through ALS8, as well as ALS with frontotemporal dementia (ALS-FTD) and ALS-FTD coupled with Parkinson's disease (ALS-FTDP). Mutations in genes encoding *angiogenin* (*ANG*) and *VEGF* and sequence variants in neurofilament genes have also been reported (for more detailed consideration of the genetics, see Gros-Louis et al. [2006]). Although all have in common disease that affects motor neurons, the nomenclature is misleading since only ALS1, ALS3, ALS6, ALS7, mutations in *angiogenin* and *VEGF*, and a small proportion of incidences of ALS8 represent the classic late-onset neurodegenerative disease with selective killing of upper and lower motor neurons leading to progressive paralysis. ALS-FTD and ALS-FTDP are likely appropriately classified as bona fide ALS, but patients have additional disease features including dementia and dystonia. For all of these disease incidences, only the genes responsible for ALS1, ALS8, and ALS-FTDP have been identified.

ALS1—Mutation in SOD1

The lion's share of work has focused on ALS1 caused by mutations in Cu/Zn superoxide dismutase (SOD1). Mutations in the *SOD1* gene are the most common form of inherited ALS, accounting for ~20% of all the familial ALS forms and corresponding to 1%–2% of all ALS cases. Since the first *SOD1* missense mutations were reported in 1993 (Rosen et al., 1993), the number of known mutations has increased to more than 114. These are distributed throughout all five exons encoding the 153 amino acid protein, with no region of the polypeptide escaping disease causing mutation. With the exception of a few instances, all *SOD1* mutations are dominant.

Sporadic and *SOD1* mutant-mediated familial ALS are clinically indistinguishable and affect the same neurons, but even within the familial forms, the time of onset and length of disease vary. The alanine-to-valine substitution at position 4 of SOD1 ($SOD1^{A4V}$) is the most prominent mutation in North America, responsible for ~50% of cases, and has a uniformly aggressive disease course with a mean survival of only 1 year after onset. Mice and rats expressing mutant forms of human or mouse SOD1

*Correspondence: dcleveland@ucsd.edu

Table 1. Genetics of Human ALS and Other Motor Neuron Diseases Sometimes Referred to as ALS

Disease	Locus	Gene (Protein/Function)	Heredity	Onset	Reference
Typical ALS					
ALS1	21q22.1	*SOD1* (Cu/Zn superoxide dismutase). Converts superoxide to water or hydrogen peroxide.	Dominant	Adult	(Rosen et al., 1993)
ALS3	18q21	Unknown	Dominant	Adult	(Hand et al., 2002)
ALS6	16q12	Unknown	Dominant	Adult	(Abalkhail et al., 2003; Ruddy et al., 2003; Sapp et al., 2003)
ALS7	20p13	Unknown	Dominant	Adult	(Sapp et al., 2003)
ALS with Dementia					
ALS-FTD	9q21–22	Unknown	Dominant	Adult ALS with frontotemporal dementia (FTD)	(Hosler et al., 2000; Ostojic et al., 2003)
ALS-FTDP	17q21.1	*MAPT* (Tau) Microtubule-associated protein.	Dominant	Adult ALS with FTD and Parkinson's Disease	(Clark et al., 1998; Hutton et al., 1998)
Atypical ALS					
ALS8	20q13.3	*VAPB* (VAMP-associated protein B). May be involved in vesicular trafficking. Interacts with itself, VAMP-A, and synaptobrevins.	Dominant	Adult. Heterogeneous disease (most cases are ALS with tremor; few cases are typical ALS; 25% of cases are late-onset spinal muscular atrophy)	(Nishimura et al., 2004a, 2004b)
Progressive lower motor neuron disease	2p13	*DCTN1* (Dynactin subunit $p150^{glued}$). Axonal transport of cellular organelles and proteins.	Dominant	Adult. Vocal-fold paralysis; weakness and muscle atrophy of hands and facial muscles	(Puls et al., 2003)
Other Motor Neuron Diseases Sometimes Referred to as ALS					
ALS2	2q33	*ALS2* (ALS2/Alsin). Endosomal dynamics. Guanine exchange factor for Rab5 and Rac1.	Recessive	Juvenile Heterogeneous disease (95% of cases are infantile-onset)	(Hadano et al., 2001; Hentati et al., 1994; Yamanaka et al., 2006; Yang et al., 2001)
ALS4	9q34	*SETX* (Senataxin). Putative DNA/RNA helicase.	Dominant	Juvenile (Recessive mutations cause ataxia-oculomotor apraxia type 2)	(Chance et al., 1998; Chen et al., 2004)
ALS5	15q15.1–q21.1	Unknown	Recessive	Juvenile	(Hentati et al., 1998)

develop progressive motor neuron degeneration (Bruijn et al., 1997; Gurney et al., 1994; Howland et al., 2002; Jonsson et al., 2004; Nagai et al., 2001; Ripps et al., 1995; Wang et al., 2003, 2005; Wong et al., 1995), and most of our knowledge on mechanisms of motor neuron pathology in ALS is from studies using these models (see below).

Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF), an established regulator of developmental, hypoxia-induced, and tumor-induced angiogenesis, gained interest as a contributor to ALS when deletion of the hypoxia response element (HRE) in the murine *VEGF* promoter resulted in ALS-like disease in mice (Oosthuyse et al., 2001). VEGF is widely expressed throughout the central nervous system (CNS) and can function as a neurotrophic factor for multiple neuronal cell types, including motor neurons (Oosthuyse et al., 2001; Van Den Bosch et al., 2004). Screening of ALS patient DNAs in promoter regions of the *VEGF* gene, including the HRE and regions known to correlate with downregulation of VEGF synthesis, found no link between HRE variants and disease (Gros-Louis et al., 2003; Lambrechts et al., 2003). Two haplotypes in other regions of the promoter, however, showed an increased risk of developing ALS in a Belgian, Swedish, and a British/Birmingham population (Lambrechts et al., 2003), but this has not been confirmed in other populations (Chen et al., 2006; Terry et al., 2004; Van Vught et al., 2005).

Angiogenin (*ANG*)

Mutations in *ANG* have also been linked to ALS, emphasizing a potential link between altered angiogenesis and motor neuron degeneration. Seven missense mutations were identified in 15 patients with familial ALS and 11 apparently sporadic ALS patients (Greenway et al., 2006). However, these mutations seem restricted to Irish and Scottish populations, as they were not found in English, Swedish, and North American populations and therefore suggest that ALS-linked *ANG* mutations are rare. The manner in which the identified mutations affect angiogenin function and provoke motor neuron disease is still unknown. Angiogenin, which is expressed within the

CNS including the motor neurons, has a known intranuclear RNase activity that can facilitate rRNA synthesis, at least in endothelial cells. Interestingly, the majority of the mutations found in ALS patients are located within the catalytic core, and one of them is in the nuclear localization signal, both predicting a loss of function of the mutated angiogenin, although this remains to be confirmed. Whether angiogenin is endowed with neurotrophic properties like VEGF, in addition to its angiogenic activity, is not yet established.

ALS8—VAMP-Associated Protein B (VAPB)

A dominant missense mutation on chromosome 20 in a set of seven kindreds has been linked to a heterogeneous disease course, with most instances of disease representing an atypical ALS that is accompanied by an unusual tremor (Nishimura et al., 2004a). About one-fourth of affected individuals in the same families develop a late-onset spinal muscular atrophy, and a small proportion of patients develop symptoms of classic ALS. All instances apparently derive from a common mutation in the gene encoding vesicle-associated membrane protein B (*VAPB*), also known as synaptobrevin-associated protein B. VAPB has been implicated in endoplasmic reticulum to Golgi transport, albeit other roles in membrane transport, including axonal transport of membrane components, are likely as well. It is unknown how the corresponding proline-to-serine substitution at position 56 affects the functional properties of the ubiquitously expressed VAPB.

Neuronal Intermediate Filaments

The genes encoding the three neurofilament subunits have long been suspected as causative for ALS because of their link with motor neuron pathology in mice and humans. Initial evidence fueling this suspicion was the realization that aberrant neurofilament accumulations are a pathological hallmark of both familial and sporadic ALS (Hirano, 1991; Hirano et al., 1984a, 1984b) and are also seen in ALS mice expressing mutant SOD1 (Bruijn et al., 1997; Dal Canto and Gurney 1994). In addition, mice with increased levels of wild-type NF-H or NF-L subunits develop age-dependent motor neuron pathology (Cote et al., 1993; Xu et al., 1993), while expression of a point mutation in NF-L at levels corresponding to that expected for dominantly inherited disease produces fatal, progressive paralysis (Lee et al., 1994).

Despite this, exhaustive screening for mutations in the three neurofilament genes in patient DNAs failed to yield conclusive linkage to either sporadic or familial ALS patients (Garcia et al., 2006), although dominant point mutations in *NF-L* have been linked to a milder motor neuron disease, Charcot-Marie-Tooth (CMT) disease (De Jonghe et al., 2001; Jordanova et al., 2003). Nevertheless, in-frame insertions or deletions within the normal array of 44 or 45 KSP repeats in the tail domain of the NF-H subunit have been reported in ~1% of sporadic ALS patients (Al-Chalabi et al., 1999; Figlewicz et al., 1994; Tomkins et al., 1998). Collectively, it is likely that these neurofilament variants are risk factors for ALS and/or a primary event with low penetrance.

A final potential contribution of neurofilaments to ALS comes from errors in the expression of an additional intermediate filament subunit, peripherin. An assembly-disrupting frameshift mutation has been reported in one sporadic ALS case (Gros-Louis et al., 2004). A proposal for the involvement of a peripherin variant, in which an intronic sequence is retained in the final mRNA that is produced during the disease course, may also contribute to human disease (Robertson et al., 2003), albeit deletion of the peripherin gene has no affect on disease course in mice having an ALS-causing SOD1 mutation (Lariviere et al., 2003).

Motor Neuron Death from Toxicity of Mutant SOD1 Not Loss of Dismutase Activity

SOD1 is an abundant, ubiquitously expressed cytosolic enzyme. Since the known activity of SOD1 is to dismutate (or convert) superoxide, a natural byproduct of respiration, to water or hydrogen peroxide, the first proposed pathogenic mechanism was the loss or diminution of this detoxifying activity. However, animals expressing dismutase active ($hSOD1^{G37R}$ [Wong et al., 1995] and $hSOD1^{G93A}$ [Gurney et al., 1994; Howland et al., 2002]) as well as inactive ($hSOD1^{G85R}$ [Bruijn et al., 1997], $mSOD1^{G86R}$ [Ripps et al., 1995], $hSOD1^{G127X}$ [Jonsson et al., 2004], $hSOD1^{Quad}$ [Wang et al., 2003], $hSOD1^{H46R}$ [Nagai et al., 2001]) forms of the enzyme develop comparable disease pathologies similar to that seen in patients, including motor neuron synapse retraction (Fischer et al., 2004; Frey et al., 2000; Pun et al., 2006), mitochondrial alterations (Higgins et al., 2003; Kong and Xu, 1998; Liu et al., 2004; Wong et al., 1995), and microglial activation (Boillée et al., 2006; Engelhardt and Appel, 1990; Hall et al., 1998; Henkel et al., 2006). Furthermore, *SOD1* gene deletion in mice does not lead to motor neuron disease (Reaume et al., 1996). In addition, deletion of the endogenous mouse SOD1 in mice expressing dismutase inactive $hSOD1^{G85R}$ does not affect disease course (Bruijn et al., 1998). Mice expressing high levels of wild-type human *SOD1* ($hSOD1^{WT}$) transgene are healthy (Gurney et al., 1994; Wong et al., 1995). Indeed, increased $hSOD1^{WT}$, accompanied by chronic elevation of dismutase activity, has either no effect on disease (Bruijn et al., 1998) or accelerates it (Deng et al., 2006; Jaarsma et al., 2000). Taken together, these experiments have argued heavily against the simple loss-of-function hypothesis.

SOD1 activity is dependent on a catalytic copper. Since free copper is highly reactive and toxic, it must be loaded onto SOD1 by a copper chaperone for SOD1 (CCS; Corson et al., 1998; Wong et al., 2000b) and subsequently held in place by a conserved disulfide bond whose formation is catalyzed by CCS (Furukawa et al., 2004). Since CCS is abundantly expressed in motor neurons (Rothstein et al., 1999) and motor neurons of *CCS*-deleted mice have an increased sensitivity to axotomy-induced death (Subramaniam et al., 2002), it was postulated that inefficient incorporation of copper into SOD1 and/or a decreased shielding of copper (due to changes in SOD1 structure as a result of mutation) could provide an opportunity for aberrant copper-dependant oxidative chemistry, yielding cellular damage and motor neuron degeneration. This hypothesis was tested using mice in which the incorporation of copper into mutant SOD1 was significantly reduced by disruption of the *CCS* gene. This did not slow disease course from either dismutase active or inactive mutants (Subramaniam

et al., 2002). Furthermore, mice in which all four copper-binding histidines have been eliminated (hSOD1^{Quad}; resulting in dismutase inactive SOD1) still develop typical ALS-like motor neuron disease (Wang et al., 2003).

Taken together, it is clear that the toxic property (or properties) acquired as a result of SOD1 mutation (1) are independent of dismutase and CCS activities and (2) can be generated without catalysis by the active site copper.

Misfolded SOD1 as a Common Feature of ALS-Causing Mutations

Aggregates are a pathological hallmark of many different neurodegenerative disorders including Alzheimer's, Parkinson's, and Huntington's diseases and ALS. Cytoplasmic protein aggregates are observed in both sporadic and familial ALS cases as well as in mutant SOD1 transgenic mice (Bruijn et al., 1997, 1998; Gurney et al., 1994; Watanabe et al., 2001; Wong et al., 1995). That said, use of the term "aggregate" is imprecise. In some instances, it refers to abnormal accumulations of intermediate filaments, including neurofilaments and peripherin, as detected by immunostaining of spinal cord tissue (Hirano et al., 1984b; Mendonca et al., 2005; Mizusawa et al., 1989; Rouleau et al., 1996; Sobue et al., 1990; Wong et al., 2000a). It has also been used to define accumulations of detergent-insoluble forms of proteins, including SOD1, that are detected by immunoblotting of filter-trappable material, as well as small SOD1- or ubiquitin-positive fibrillar inclusions in spinal cord sections (Deng et al., 2006; Furukawa et al., 2006; Johnston et al., 2000; Jonsson et al., 2004, 2006; Wang et al., 2003, 2005). Detergent-insoluble species are detectable only in affected tissues of mutant SOD1 mice and are most prominent at symptomatic stages (Furukawa et al., 2006; Jonsson et al., 2006). A propensity to form aggregates following synthesis of mutant SOD1 in primary cells is selective to motor neurons, as aggregates were not produced in dorsal root ganglion (DRG) or hippocampal neurons expressing similar levels of mutants (Durham et al., 1997). The most misfolded unstable SOD1 mutants (with the shortest in vivo half-lives) are most prone to aggregation (Wang et al., 2003).

A very curious and unexplained aspect of disease is that the very unstable mutant hSOD1^{A4V} (Sato et al., 2005), which provokes a very aggressive disease course in humans, neither induces aggregates nor ALS-like disease in mice, except in the context of high levels of hSOD1^{WT} (Deng et al., 2006). Similarly, increased hSOD1^{WT} accelerated disease from a second unstable mutant hSOD1^{L126Z}. In both cases, accelerated toxicity may be mediated by stabilization of the mutant via heterodimerization with hSOD1^{WT} protein. Indeed, in both cases, increased levels of hSOD1^{WT} generated detergent-insoluble forms of SOD1 that were not seen in spinal cord extracts of hSOD1^{A4V} or hSOD1^{L126Z} mice alone.

Are SOD1 Aggregates Toxic?

Regardless of their definition or composition, the contribution of aggregates to motor neuron toxicity remains to be established. Indeed, it is unknown whether any of these aggregates are indeed toxic. As introduced in Figure 1, proposals include aggregate-mediated inhibition of the proteasome machinery, decreased chaperone activity, deregulation of organelle function including Golgi, endoplasmic reticulum, and mitochondria, and axonal transport defects possibly linked to aberrant accumulations of intermediate filaments.

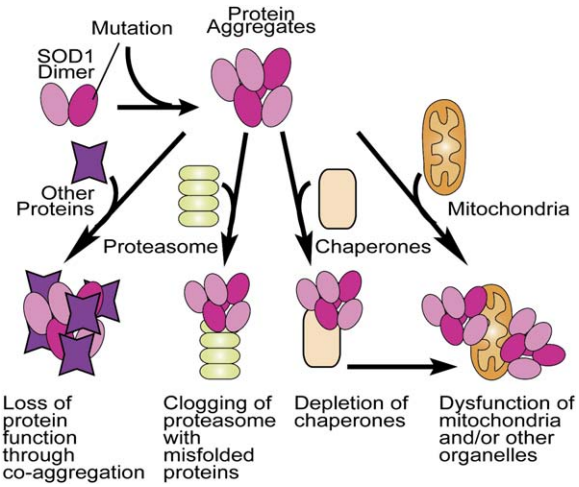

Figure 1. Proposed Toxicities of ALS-Causing SOD1 Protein Aggregates

Cell machinery that might be affected by misfolded, mutant SOD1 includes coaggregation of essential cytoplasmic components, poisoning of the proteasome thereby inhibiting timely degradation of many cellular proteins, saturation of cytoplasmic chaperones that catalyze essential protein folding and refolding, and damaging mitochondria by aggregation onto the cytoplasmic surface and/or transport into the mitochondrial intermembrane space.

Aggregates found in ALS patients, as well as mouse models, contain ubiquitin (Ince et al., 1998; Wang et al., 2003; Watanabe et al., 2001), a protein adduct which typically targets proteins for disposal via the proteasome. Misaccumulation of ubiquitinated, misfolded proteins might adversely affect the proteasome machinery and impair normal protein degradation. Indeed, the degradation of SOD1 itself and aggregates of SOD1 is proteasome mediated (Basso et al., 2006; Niwa et al., 2002; Urushitani et al., 2004; Urushitani et al., 2002). While proteasome malfunction has been implicated in motor neuron death, it is not yet established if it is a cause or consequence of aggregate formation. Contradictory results have been reported. In hSOD1^{G93A} mice which accumulate mutant protein to high levels, proteasome activity is downregulated in the lumbar spinal cord well before the development of symptoms (Kabashi et al., 2004), while in a different line of mice with lower accumulated levels of the same mutant, increased proteasome activity in the spinal cord has been reported at symptomatic stages (Puttaparthi and Elliott, 2005).

Protein misfolding is a probable initiator of aggregate formation. Thus, many efforts have examined the protein folding chaperone machinery, the heat-shock proteins (HSPs), in the context of disease. In spinal cord extracts of presymptomatic ALS mice, an overall decrease of chaperone activity has been reported which persists throughout disease course, and multiple recombinant SOD1 mutants inhibit chaperone function in vitro (Bruening et al., 1999; Tummala et al., 2005). Paradoxically, some HSPs, such as αB-crystallin and Hsp27 are

elevated in the spinal cords of hSOD1^{G37R} and hSOD1^{G93A} mice, but Hsp27 is predominantly present in glial cells in late disease stages (Vleminckx et al., 2002; Wang et al., 2003). Hsp70, Hsp40, and Hsp90 are also elevated but only in the spinal cords of hSOD1^{G85R} mice (Liu et al., 2005; Vleminckx et al., 2002; Wang et al., 2003).

Motor neurons have a high threshold for induction of the stress response, at least in vitro (Batulan et al., 2003), which may contribute to their selective vulnerability in ALS. Mutant SOD1-mediated depletion of HSPs is a plausible possibility given that Hsp70 and Hsp25 preferentially bind mutant SOD1 (Okado-Matsumoto and Fridovich, 2002). Expression of several different HSPs (Hsp70, Hsp40, Hsp27) in cultured cells and primary motor neurons decreases aggregate content and apoptosis and improves axonal outgrowth (Bruening et al., 1999; Patel et al., 2005; Takeuchi et al., 2002). Unfortunately, applying this strategy in vivo by increased expression of Hsp70 in four different mutant SOD1 mouse lines did not ameliorate disease or pathology (Liu et al., 2005). Induction of a broader stress response extended life span in a small cohort of hSOD1^{G93A} mice after treatment with arimoclomol, a drug which induces the phosphorylation-mediated activation of the HSP inducing factor HSF-1, thereby leading to increased levels of Hsp70 and Hsp90 in spinal cords (Kieran et al., 2004). Interestingly, missense mutations in the gene encoding Hsp27 have been identified in a number of families with distal hereditary motor neuropathies and CMT neuropathies (Evgrafov et al., 2004).

Mitochondrial Dysfunction as a Proximal Cause of Motor Neuron Death

A common feature of many neurodegenerative diseases is damage to mitochondria that contributes to the degenerative phenotype. Mitochondria were implicated as a possible target for toxicity in ALS by histopathological observations of vacuolated and dilated mitochondria with disorganized cristae and membranes in the motor neurons (and muscle) of both sporadic and familial ALS patients (Afifi et al., 1966; Hirano et al., 1984a, 1984b). Mitochondrial defects have been reported in spinal cords and muscle biopsies of patients ranging from histopathological observations to impaired mitochondrial respiration and increased levels of uncoupling proteins (Chung and Suh, 2002; Dupuis et al., 2003; Echaniz-Laguna et al., 2002; Vielhaber et al., 1999; Wiedemann et al., 2002). However, since all patients tested were obligatorily symptomatic, it is difficult to determine if these defects are primary or secondary to muscle atrophy.

Mitochondrial abnormalities, especially multimembrane-containing vacuoles, are observed in the motor neurons of mice that develop disease from accumulation of dismutase active mutants (hSOD1^{G37R} [Wong et al., 1995] and hSOD1^{G93A} mice [Dal Canto and Gurney, 1994; Higgins et al., 2003; Kong and Xu, 1998]) at very early, presymptomatic stages (prior to any detectable motor neuron loss or other observable damage (Higgins et al., 2003; Kong and Xu, 1998; Wong et al., 1995). It has been proposed that vacuole formation is due to expansion of the mitochondrial intermembrane space and consequent distention of mitochondrial membranes (Higgins et al., 2003). However, vacuoles inferred to derive from degeneration of mitochondria have also been described in mice with very high accumulated levels of hSOD1^{WT} protein (Jaarsma et al., 2000, 2001) which do not develop disease and are absent in mice that develop disease from dismutase inactive mutants (Bruijn et al., 1997; Jonsson et al., 2006). Thus, vacuolation cannot be a primary/common pathogenic mechanism for motor neuron degeneration.

A proportion of the predominantly cytosolic SOD1 localizes to mitochondria in certain contexts. In both rodent models and patient samples, mutant SOD1 is present in fractions enriched for mitochondria derived from affected, but not unaffected tissues (Bergemalm et al., 2006; Deng et al., 2006; Liu et al., 2004; Vijayvergiya et al., 2005). Mitochondrial localization of SOD1 has been confirmed by electron microscopy in both isolated mitochondria (Liu et al., 2004) and motor neurons in situ (Higgins et al., 2002; Sasaki et al., 2004). SOD1 mutants that cause disease at the lowest accumulated levels (hSOD1^{G85R} and hSOD1^{G127X}; all dismutase inactive) have the highest relative proportions that are mitochondrially associated (Liu et al., 2004). There is disagreement in defining the submitochondrial compartment(s) with which SOD1 is localized (or potentially aggregated). Mutant SOD1 has been reported in both the intermembrane space and the matrix, as well as both the inner and outer membranes of spinal cord and brain mitochondria (Bergemalm et al., 2006; Deng et al., 2006; Higgins et al., 2002; Liu et al., 2004; Pasinelli et al., 2004; Vijayvergiya et al., 2005). While these apparent contradictions remain to be resolved, all reports agree that SOD1 mutant proteins of divergent biochemical characteristics localize to mitochondria, consistent with a common contribution to pathogenesis.

Key questions are unresolved. Is there some intrinsic feature of spinal cord mitochondria which permits SOD1 association and/or increases mitochondrial vulnerability to mutant SOD1? Alternatively, is it the cytoplasmic components of chaperone or proteasome activities which confers some selectivity and facilitates mitochondrial association (Figure 1)? In which cell type(s), if any, does mitochondrial association of the mutant play a central role in pathogenesis? Of relevance here, the endogenous wild-type SOD1 protein is largely excluded from spinal cord mitochondria, but all human SOD1 mutants examined to date are mitochondrially associated. The same human mutant proteins are largely excluded from similar preparations of liver mitochondria (Liu et al., 2004) despite a proportion of endogenous mouse SOD1 localized to the intermembrane space of those mitochondria (Liu et al., 2004; Okado-Matsumoto and Fridovich, 2002). This discordance highlights that not all mitochondria are created equally, as supported by recent proteomic efforts profiling mitochondrial heterogeneity across tissues (Kislinger et al., 2006; Mootha et al., 2003).

How mutant SOD1 affects mitochondrial function is not yet clear, but differences in protein composition between mutant and nonmutant mitochondrial populations have been reported (Fukada et al., 2004; Kirby et al., 2005; Lukas et al., 2006). It is important to note that the mitochondrial genome itself encodes for only thirteen of the estimated 1500 proteins required for

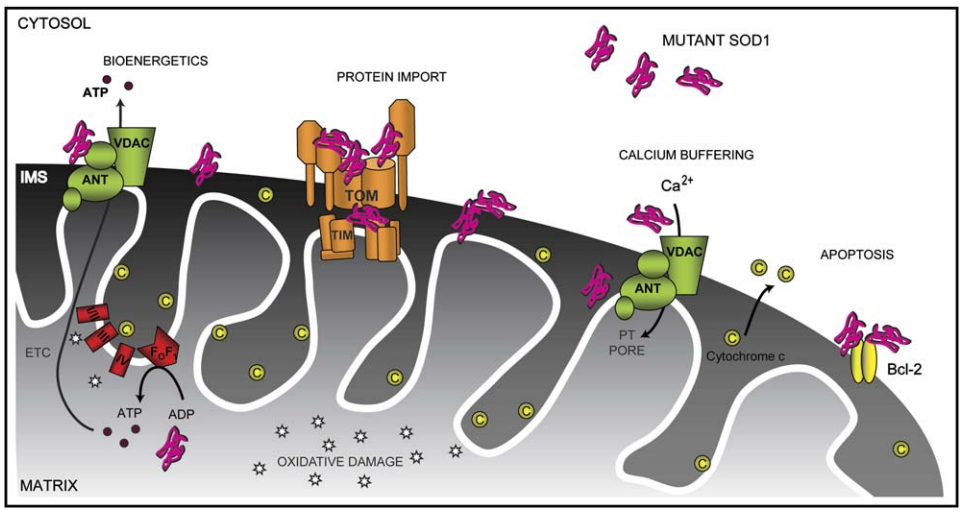

Figure 2. Mutant SOD1-Mediated Damage to Mitochondria

Mutant SOD1 is preferentially imported into or deposited onto mitochondria in affected tissues. The mechanisms by which mutant protein might damage mitochondrial function are drawn. Mutant SOD1 may interfere with the elements of the electron transport chain, thereby disrupting ATP-generating oxidative phosphorylation. Mutant SOD1 may also disrupt mechanisms by which mitochondria buffer cytosolic calcium levels. Mutant SOD1 aggregates may interfere with components of mitochondrial-dependent apoptotic machinery, such as Bcl-2, thereby triggering premature activation of an apoptotic cascade including cytochrome c release into the cytosol. Mutant SOD1 may indirectly affect similar pathways linked to mitochondria by physically blocking the protein import machines, TOM and TIM. Oxidative damage incurred by various mitochondrial proteins may also contribute to overall mitochondrial dysfunction. Collectively, these mechanisms (or a combination thereof) are predicted to disturb cellular homeostasis (within glial and/or motor neurons), ultimately triggering motor neuron death.

function. The majority of mitochondrial proteins are nuclear encoded and thus are synthesized in the cytosol and subsequently transported into mitochondria. Import of the majority of mitochondrial proteins is accomplished by membrane-spanning multisubunit translocators of the outer and inner membrane (TOM and TIM, respectively). Mutant SOD1 associated with or aggregated onto the mitochondrial surface could impede the import machinery (Liu et al., 2004).

Many aspects of the electron transport chain, which generates ATP via oxidative phosphorylation, have been reported as either unchanged or altered, although there is little or no consensus as to whether these activities are accentuated or attenuated. For example, ATP synthesis has been reported as unchanged in aged hSOD1^{G85R} mice (Damiano et al., 2006) or depleted in late symptomatic hSOD1^{G93A} mice (Mattiazzi et al., 2002), or ATP levels are depleted in presymptomatic mice (Browne et al., 2006). Creatine, which extended survival in hSOD1^{G93A} mice by alleviating presumed energy deficits (Browne et al., 2006; Klivenyi et al., 1999), was ineffective in human clinical trials (Groeneveld et al., 2003; Shefner et al., 2004). Complex I activity has been reported as elevated (Bowling et al., 1993; Browne et al., 1998), reduced (Jung et al., 2002; Kirkinezos et al., 2005; Mattiazzi et al., 2002), and unchanged (Damiano et al., 2006). Complex IV activity is reportedly reduced, but only at very late disease stages (Kirkinezos et al., 2005; Mattiazzi et al., 2002). Early impairment in mitochondrial calcium-buffering capacity in mutant SOD1 spinal cord prior to symptoms and only in disease-relevant tissues in two different mutant SOD1 models (Damiano et al., 2006) is perhaps the most persuasive of the efforts, since mitochondrial control of calcium homeostasis is fully compatible with excitotoxic mechanisms that may be central to neuronal damage (see Figures 2 and 3 and below).

Mitochondria: The Gatekeepers of Cell Death

Mitochondria are the gatekeepers of apoptosis, with opening of the permeability transition pore and release of cytochrome c central to the cascade of caspase activation. A final apoptotic mechanism is probably a central aspect of neuronal death, as activation of the executioner caspase-3 is found in mouse models contemporaneous with neuronal death (Li et al., 2000; Pasinelli et al., 2000) and lowered levels of the antiapoptotic Bcl-2 have been reported in spinal cord motor neurons of ALS patients (Ekegren et al., 1999; Mu et al., 1996) and mutant SOD1 mice (Gonzalez de Aguilar et al., 2000; Vukosavic et al., 2000). Intriguingly, it has recently been demonstrated that caspase-3 activation in glial cells proteolytically inactivates the glutamate transporter EAAT2 (Boston-Howes et al., 2006). Since EAAT2 is selectively lost during the disease process and is considered to be a main participant in excitotoxicity (which can trigger additional mitochondrial dysfunction), this provides an attractive mechanism by which to unite mitochondrial dysfunction with excitotoxicity (see later).

An alternate mechanism, in which SOD1 is proposed to interact with Bcl-2 and possibly interferes with Bcl-2 antiapoptotic activity (Pasinelli et al., 2004), is attractive but has recently been disputed (Gould et al., 2006). However, both constitutively increased expression of Bcl-2 (Kostic et al., 1997) and virally driven overexpression of Bcl-2 in motor neurons (Azzouz et al., 2000) protect motor neurons, delaying disease onset but not progression. Bcl-2 is unable to similarly rescue motor neuron death in other models, such as *wobbler* and transgenic

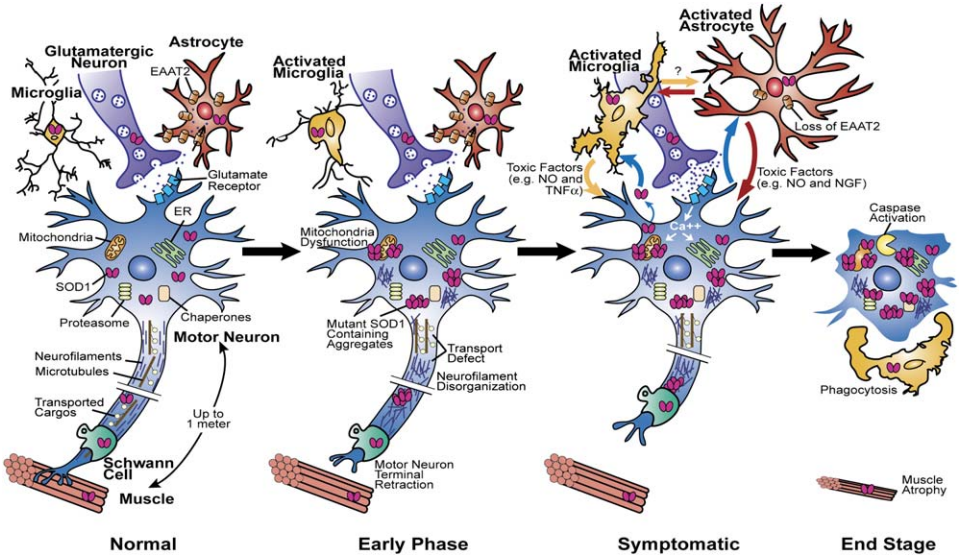

Figure 3. Schematic of the Evolution of Motor Neuron Degeneration and Glial Activation during the Course of SOD1 Mutant-Initiated ALS Disease

Four stages are defined (normal, early phase, symptomatic, and end stage). Toxicity is non-cell-autonomous, produced by a combination of damage incurred directly within motor neurons that is central to disease initiation and damage within nonneuronal neighbors, including astrocytes and microglia, whose actions amplify the initial damage and drive disease progression and spread. Selective vulnerability of motor neurons to ubiquitously expressed mutant SOD1 is determined by the unique functional properties of motor neurons (e.g., they are very large cells with large biosynthetic loads, high rates of firing, and respond to glutamate inputs) and damage to their supporting cells in the neighborhood.

mice expressing a point mutation in the *NF-L* gene (Coulpier et al., 1996; Houseweart and Cleveland, 1999). Moreover, consistent with a similar outcome in which intracerebroventricular (ICV) infusion of a broad spectrum caspase inhibitor (Li et al., 2000) slowed mutant SOD1-mediated neuronal death, disease onset was also delayed in the absence of Bax, but progression was unaffected (Gould et al., 2006), casting doubt on the utility of antiapoptotic therapies.

Loss of the Neuromuscular Synapse as the First Cellular Phenotype in ALS

The axon is the only means by which a motor neuron communicates with its downstream target muscle, both sending output information and receiving signaling inputs from the muscle and other cells. Connection to the muscle at the neuromuscular junction is lost in ALS mouse models long before motor neuron degeneration or death and the initiation of symptoms (Fischer et al., 2004; Frey et al., 2000; Kong and Xu, 1998; Pun et al., 2006). It has been long established in humans (Kawamura et al., 1981) and in mice (Bruijn et al., 1997; Wong et al., 1995) that it is the motor neurons that generate large caliber (>5 μm) myelinated axons that are selectively vulnerable in ALS. This important distinction has now been refined with the demonstration that different types of motor neurons, innervating different subsets of muscle fibers, have different susceptibilities to mutant SOD1 toxicity. Specifically, in two different mutant SOD1 models, the fast-fatiguable motor neurons were shown to be affected first, with denervation of their target muscles well before symptoms. Retraction of the fast-fatigue-resistant motor neurons followed next, with the slow type partially resistant to mutant SOD1 and actually attempting to reinnervate previously denervated regions (Frey et al., 2000; Pun et al., 2006).

A spontaneous dominant mouse mutant *Wld*^{s} (Wallerian degeneration slow) significantly delays the degeneration of axonal segments located distal of an axonal cut (axotomy; Glass et al., 1993; Perry et al., 1990). This is due to a unique fusion protein containing the first 70 residues of the ubiquitination factor UFD2/E4 joined to mononucleotide adenylyltransferase (Nmnat), an enzyme that facilitates nicotinamide adenine dinucleotide (NAD) synthesis (Conforti et al., 2000; Mack et al., 2001). Despite conferring robust axonal protection in neuropathy mouse models, including the peripheral neuropathy model (*pmn*/*pmn*; Ferri et al., 2003) and a model of myelin-related axonopathy (P0-deficient; Samsam et al., 2003), *Wld*^{s} failed to provide a benefit in three different mutant SOD1 models (Fischer et al., 2005; Vande Velde et al., 2004).

Damage within the Axon: Compromised Axonal Transport in ALS

Motor neurons are among the most asymmetric cells in nature, extending axons in humans that can be more than a meter in length. Alterations in axonal structure are well documented in both patients (Hirano et al., 1984a, 1984b; Kawamura et al., 1981) and mutant SOD1 mice (Bruijn et al., 1998; Kong and Xu, 1998; Wong et al., 1995), especially the inappropriate misaccumulation of neurofilaments, the most abundant structural components of large myelinated axons. Neurofilaments are obligate heteropolymers of NF light (NF-L), NF medium (NF-M), and NF heavy (NF-H) subunits and interestingly, transgenic mice expressing a point mutation in NF-L develop motor neuron disease (Lee et al., 1994). Neurofilament accumulations are seen early in mutant SOD1 mice (Kong and Xu, 1998). Removal of all axonal neurofilaments by deletion of NF-L substantially prolonged survival of hSOD1^{G85R} and hSOD1^{G37R} mice

(Nguyen et al., 2001; Williamson et al., 1998), as did removal of most axonal neurofilaments by excessive levels of NF-H (Couillard-Despres et al., 1998; Kong and Xu, 2000; Nguyen et al., 2001). Since neurofilament-dependent slowing of slow axonal transport is itself directly dependent on the phosphorylation state of neurofilament tail domains (Ackerley et al., 2003), the increased survival benefit likely derives primarily from reducing the neurofilament-dependent burden on the axonal transport machinery. This was demonstrated to be the case by use of gene replacement to remove the phosphorylated "tail" domains of NF-M and NF-H that normally provide intra-axonal crosslinking of adjacent filaments. Absence of those tail domains sharply slows SOD1 mutant-induced disease (Lobsiger et al., 2005). Consistent with this, a presymptomatic deficit of slow axonal transport has been described in mutant SOD1 mice (Ackerley et al., 2003; Williamson and Cleveland, 1999, Zhang et al., 1997).

Additional evidence for a link between transport and motor neuron disease arose from the discovery of a mutation in the $p150^{Glued}$ subunit of dynactin in a family affected with a slowly progressive, autosomal dominant form of lower motor neuron disease (Puls et al., 2003). $p150^{Glued}$ is responsible for providing processivity by bridging between microtubules and cytoplasmic dynein (Vaughan and Vallee, 1995; Waterman-Storer et al., 1995), a motor powering retrograde axonal transport along microtubules (Goldstein and Yang, 2000). Mice heterozygous for either of two point mutations in dynein have mispositioned hindlimbs, a phenotype reflected in the names of the mutations, Legs at odd angles (*Loa*) and Cramping1 (*Cra1*; Hafezparast et al., 2003). Surprisingly, introducing either the *Loa* (Kieran et al., 2005) or *Cra1* (Teuchert et al., 2006) mutation to $hSOD1^{G93A}$ mice significantly improves survival. This unexpected amelioration of SOD1 mutant toxicity by a mutation expected to alter retrograde axonal transport is indeed a conundrum that remains to be resolved.

Further genetic evidence implicating axonal transport defects as a bona fide pathogenic mechanism in motor neuron degeneration stem from additional mutations in multiple genes relevant to transport in both human disease and mouse models. These include loss of the kinesin motor protein KIF1Bβ as causative for CMT type 2A (Zhao et al., 2001), loss of KIF5A in hereditary spastic paraplegia (Reid et al., 2002), and loss of KIF21A in a rare disorder affecting the oculomotor nerve (Yamada et al., 2003). Mutations in genes involved in transport of membranous vesicles include the ALS8 gene *VAPB* (Nishimura et al., 2004b), *Rab7* in CMT type 2B (Verhoeven et al., 2003), and *Vps54* (vacuolar-vesicular protein sorting 54) which is responsible for cervical motor neuron degeneration in the *wobbler* mouse (Schmitt-John et al., 2005).

The Neighborhood Matters: Non-Cell-Autonomous Death of Motor Neurons

Although progressive paralysis in ALS arises from degeneration and death of motor neurons, evidence from several directions has converged to demonstrate that toxicity is non-cell-autonomous. That is, toxicity to motor neurons derives from damage developed within cell types beyond the motor neurons. Consistent with this, the known inherited forms are caused by mutations in genes that are ubiquitously expressed (*SOD1* and *VAPB*) or expressed in multiple cells types (*VEGF* and *ANG*). The initial evidence that damage within more than one cell type was required for disease came from expression of mutant SOD1 only within motor neurons (Lino et al., 2002; Pramatarova et al., 2001) or astrocytes (Gong et al., 2000). None of these efforts produced motor neuron degeneration or death (albeit the expression of mutant SOD1 selectively within motor neurons might have been at levels too low to initiate disease).

More definitive evidence emerged from construction and analysis of chimeric mice that were mixtures of hSOD1 mutant-expressing cells and normal cells (Clement et al., 2003). Some animals with only a small minority of normal cells escaped disease, despite having high proportions of mutant motor neurons. These efforts conclusively demonstrated that expression of mutant SOD1 within individual motor neurons, even at levels that cause early onset, rapidly progressing disease when expressed ubiquitously, is not sufficient to provoke cell-autonomous degeneration or death of individual motor neurons of comparable ages.

Construction and analysis of mice carrying a deletable ("floxed") mutant *SOD1* gene that can be excised by the action of the Cre recombinase has identified differential contribution of mutant damage within motor neurons and nonneuronal neighbors in triggering disease onset and progression (Boillée et al., 2006). Excision of the floxed mutant *SOD1* gene exclusively within motor neurons (by action of Cre recombinase expressed under control of the motor-neuron-specific promoter Islet-1) extended survival by slowing onset and an early phase of disease progression. Similar findings by altering components expressed only within neurons (i.e., gene disruption to remove all neurofilaments [Williamson et al., 1998] or gene replacement to alter axonal structure and volume after elimination of the tail domains of the NF-M and NF-H subunits [Lobsiger et al., 2005]) provide extended survival of SOD1 mutant mice but only by slowing disease onset.

In contrast, diminishing mutant SOD1 levels within microglia and peripheral macrophages (using a *Cre* transgene with a CD11b promoter that is expressed only within the microglia and peripheral macrophage lineages) had little effect on early disease but sharply slowed later disease progression, extending overall survival by 99 days. Similarly, in a complementary approach the entire myeloid lineage was replaced by transplantation of normal bone marrow cells into SOD1 mutant mice that were themselves unable to synthesize their own myeloid cells due to deletion of the transcription factor PU.1 (Beers et al., 2006). Transplantation at birth with $hSOD1^{G93A}$ mutant-expressing myeloid cells (which populate both the CNS and the periphery) produced onset and survival typical of the $hSOD1^{G93A}$ mutant line. Replacement of the microglial, monocyte, and macrophage lineages with normal cells had no effect on disease onset but slowed disease progression after onset.

Thus, both approaches demonstrated that mutant SOD1 within macrophages/microglial cells accelerates disease progression, while mutant action within the motor neurons is a primary determinant of onset and

early disease. Importantly, introducing mutant SOD1-expressing microglial cells into control animals did not give rise to motor neuron disease, demonstrating that mutant SOD1-expressing macrophages/microglial cells themselves are not sufficient to cause motor neuron death.

It should be emphasized that a contribution of peripheral macrophages to accelerating disease progression cannot be excluded. Indeed, a proportion of macrophages/microglial cells in the spinal cord of mutant SOD1 mice have been shown to enter from the periphery during the course of disease in mice (albeit this contribution seems minor [Solomon et al., 2006]). There is disagreement whether replacing all of the peripheral, but only a few of the spinal cord, myeloid derived cells by bone marrow transplantation following irradiation is beneficial in slowing disease (Corti et al., 2004; Solomon et al., 2006). However, there is no uncertainty that replacing both the peripheral and CNS microglial pools slows disease progression after onset (Beers et al., 2006).

Contributions to toxicity developed within astrocytes, the myelinating Schwann cells, or the target muscles have not yet been established, although the "floxed" mutant transgene approach is well suited for such tests.

Targets for Therapy: Microglial Cells as Determinants of Disease Progression

Microglial cells, the macrophages of the CNS, have been long suspected as central components in neurodegenerative diseases where their role may include secretion of trophic or toxic molecules. Their role in neuronal degeneration during development is well established (Marin-Teva et al., 2004). In ALS, microglial activation has been described in the brain and spinal cord of patients (Engelhardt and Appel, 1990; Henkel et al., 2004; Kawamata et al., 1992; McGeer et al., 1991; Troost et al., 1993; Turner et al., 2004) and in the spinal cord of different mutant SOD1 mouse models (Hall et al., 1998; Henkel et al., 2006; Kriz et al., 2002). The microglial reactivity is initiated before motor neuron loss (Henkel et al., 2006). In addition, increased numbers of dendritic cells, potent antigen-presenting cells implicated in the immune response, have been reported in spinal cords of ALS patients and hSOD1^{G93A} mice during the disease course (Henkel et al., 2006; Henkel et al., 2004).

Several groups have tried to treat motor neuron disease by using minocycline, a tetracycline derivative previously shown to inhibit microglial activation (Yrjanheikki et al., 1999). Minocycline was potent in increasing survival of ALS mice and reduced microglial activation (Kriz et al., 2002; Van Den Bosch et al., 2002; Zhu et al., 2002). The primary effect of minocycline treatment initiated in very young mice is a delay in disease onset (Zhu et al., 2002), while administration closer to disease onset results in a slowing of disease progression after onset (Kriz et al., 2002). Regardless, both treatment paradigms provided a comparable extension of survival (Kriz et al., 2002). Since minocycline also exerts an antiapoptotic property to neurons (Zhu et al., 2002), it is unclear in which cells the minocycline effect was active. Either way, supported by its beneficial effect in ALS mice, minocycline has been proposed for clinical trial (Gordon et al., 2004; Pontieri et al., 2005).

Cyclooxygenase-2 (COX-2), produced in abundance by microglia and other inflammatory cells (but also by neurons and astrocytes), plays a key role in stimulating production of proinflammatory cytokines, and COX-2 expression is induced in spinal cords of ALS patients (Yasojima et al., 2001; Yiangou et al., 2006). In mice, use of a COX-2 inhibitor (celecoxib) prolonged survival by slowing disease onset (Drachman et al., 2002) but disappointingly did not alter progression after onset. Subsequent test of celecoxib in a human trial failed to provide a benefit in ALS (Cudkowicz et al., 2006).

Additional evidence implicating microglia in pathogenesis of ALS arose from forcing activation of the immune system using chronic administration of lipopolysaccharide (LPS), a well-known microglial activator. Such treatment exacerbated SOD1 mutant-mediated disease in mice (Nguyen et al., 2004). Similarly, inducing microglial activation by deleting the fractalkine receptor, a cytokine receptor expressed by microglia, mildly accelerated neuronal loss in mutant SOD1 mice (Cardona et al., 2006). Increased levels of several proinflammatory factors have been described in the spinal cords of mutant SOD1 mice even before motor neuron loss (Elliott, 2001; Hensley et al., 2003; Yoshihara et al., 2002). Cerebrospinal fluid (CSF) and serum of ALS patients also contain increased levels of inflammation related factors, including complement proteins and monocyte chemoattractant protein-1α (MCP-1α; Goldknopf et al., 2006; Simpson et al., 2004).

One particularly interesting cytokine upregulated in mutant SOD1 mouse spinal cords which could play a role in motor neuron degeneration is tumor necrosis factor-α (TNFα). TNFα has been shown to be produced in higher levels by adult hSOD1^{G93A} microglial cells when stimulated with LPS compared to nontransgenic microglial cells (Weydt et al., 2004). Administration of a TNFα antagonist yielded a mild increase in survival in mutant SOD1 mice (West et al., 2004). A direct toxic effect of activated microglial cells on motor neurons has been shown in a coculture system and seemed to implicate nitric oxide (Zhao et al., 2004). Microglial cells could therefore be an important player in a Fas-ligand (FasL)-induced apoptosis pathway within motor neurons that is driven by nitric oxide synthesis (discussed in more detail below). In addition, mutant SOD1, which has now been reported to be released by motor neurons, is a potent activator of microglial cells (Urushitani et al., 2006), emphasizing the likely crosstalk between motor neurons, microglial cells, and potentially other nonneuronal cells that may cooperate to drive disease progression.

An Outside-In Cascade Triggering a Cell-Death Pathway Intrinsic to the Motor Neuron

At least a partial explanation for the selectivity of motor neurons to SOD1 mutant toxicity has arisen from identification of a motor-neuron-specific cell-death pathway. Not surprisingly, embryonic motor neurons extracted from spinal cords of mutant SOD1 mice are more susceptible to toxic insults (Kruman et al., 1999; Raoul et al., 2002; Spalloni et al., 2004b; Van Den Bosch et al., 2004) and have modified electrophysiology and altered excitability (Pieri et al., 2003; Spalloni et al., 2004a) compared to neurons from normal mice or mice expressing high levels of hSOD1^{WT} protein. These neurons

also have what has been proposed as a motor-neuron-specific cell-death pathway downstream of the Fas death receptor. Increased susceptibility to death triggered by the Fas receptor requires nitric oxide, the Fas death domain-associated protein Daxx, and p38 kinase activation, which subsequently drives nitric oxide production, thus generating a feed-forward amplification loop (Raoul et al., 1999, 2002, 2006). Cultured motor neurons from transgenic mutant SOD1 mice have an increased susceptibility to activation of this pathway, which is apparently also activated in presymptomatic ALS mice (Raoul et al., 2002, 2006; Wengenack et al., 2004). Given the acceleration of disease by mutant microglia, it is plausible that SOD1 mutant-induced release of nitric oxide by microglia drives this death cascade within motor neurons.

Astrocytes in ALS: Gatekeepers of Synaptic Glutamate and Excitoxicity

Astrocytes are essential partners of motor neurons, providing them with trophic support and mediating rapid recovery of synaptic glutamate through the action of the glial glutamate transporter EAAT2 (also referred to as GLT-1 in rodents). Astrocytes insert finger-like processes loaded with this transporter into and surrounding synapses, thereby facilitating rapid removal of synaptic glutamate following its release from the presynaptic terminal (as detailed in Figure 3). Glutamate-mediated excitotoxicity can induce neuronal damage and death as a consequence of repetitive firing, which in turn drives calcium entry through calcium-permeable AMPA glutamate receptors. Within the spinal cord, preventing glutamate-driven excitotoxicity is primarily the job of astrocytes. Motor neurons have an inherently high sensitivity to excitotoxicity since their glutamate receptors have a lower proportion of the GluR2 subunit (Van Damme et al., 2002) relative to many other types of neurons. Regulation of the Ca^{2+} permeability properties of GluR2 is due to posttranscriptional RNA editing, with only AMPA receptors containing edited GluR2 resistant to Ca^{2+} entry. Already with low GluR2 levels, the efficiency of mRNA editing is reduced in the spinal cord motor neurons of ALS patients compared to controls (Kawahara et al., 2004; Takuma et al., 1999). Altered efficiency of editing was not found in hSOD1^{G93A} or hSOD1^{H46R} rats (Kawahara et al., 2006), thereby demonstrating that change in mRNA editing is not required for familial disease.

Astrocytes also respond to damage in many settings by what is referred to as activation. This includes an increase in assembly of their intermediate filaments (assembled from glial fibrillary acidic protein, GFAP) and an increase in the number and size of processes extended from the cell body (Figure 3). Astrocyte activation is seen in spinal cords of ALS patients and SOD1 mutant mice (Hall et al., 1998; Levine et al., 1999; Schiffer et al., 1996). Indeed, for mice expressing the dismutase inactive mutant hSOD1^{G85R}, a very prominent early pathology that increases markedly during disease course is the presence of SOD1-containing inclusions within activated astrocytes (Bruijn et al., 1997).

Altered glutamate handling is one of the few firm mechanistic links between sporadic and SOD1 mutant-caused ALS. Diminished glutamate transport has been reported in synaptosomes obtained from affected CNS tissues of ALS patients (Rothstein et al., 1992), and levels of EAAT2 are reduced in the motor cortex and spinal cord of ALS patients (Fray et al., 1998; Maragakis et al., 2004; Rothstein et al., 1995; Sasaki et al., 2000), spinal cords of mutant SOD1 mice (Bruijn et al., 1997) and rats (Howland et al., 2002), as well as in a model of Sindbis virus-induced motor neuron disease (Darman et al., 2004). This is functionally of consequence for familial ALS in that hSOD1^{G93A} mice heterozygous for EAAT2 develop earlier-onset disease (Pardo et al., 2006), while drugs that increase EAAT2 activity extend survival (Ganel et al., 2006; Rothstein et al., 2005). Indeed, screening of FDA-approved drugs for those that could elevate EAAT2 activity has identified a CNS-penetrating β-lactam antibiotic, ceftriaxone, as a transcriptional inducer that modestly extends survival in hSOD1^{G93A} mice (Rothstein et al., 2005). An inhibitory effect on quantal glutamate release is also believed to underlie the mild slowing of disease course in human ALS from the only FDA-approved treatment (riluzole; Doble, 1996).

Additional implication for an astrocytic contribution in ALS is demonstration in cell culture that activated astrocytes induce embryonic motor neuron degeneration via their production of NGF, which in the presence of low concentrations of nitric oxide can induce apoptosis of motor neurons through the p75^{NTR} receptor. Indeed, relative to resting astrocytes, activated astrocytes in the spinal cord of hSOD1^{G93A} mice have increased NGF synthesis (Pehar et al., 2004).

Muscle Involvement in ALS: Targets for Therapy?

Among the earliest events in the human ALS- and SOD1-mediated disease is withdrawal of the motor axons from the neuromuscular synapse (Fischer et al., 2004; Frey et al., 2000; Pun et al., 2006; Schaefer et al., 2005). This denervation generates the progressive paralysis of ALS, a consequence of which is muscle atrophy (Cifuentes-Diaz et al., 2001). Mutant SOD1 is also expressed by muscle, but whether its presence there contributes to pathogenesis is not yet established.

A test of whether enhanced muscle mass and strength per se can be beneficial in ALS has been attempted by repetitive injection into hSOD1^{G93A} mice of an antibody to myostatin, a secreted protein whose action inhibits muscle growth. Initiated before disease onset, this produced enhanced muscle mass initially, but the effect on limb muscles was short lived, yielding neither a survival benefit nor preservation of muscle mass throughout disease progression (Holzbaur et al., 2006).

On the other hand, muscle hypertrophy induced by agents such as insulin-like growth factor-1 (IGF-1) or growth hormone (Dobrowolny et al., 2005; Kaspar et al., 2003, 2005) has led to significant life extensions in ALS transgenic mice. For the IGF-1 studies, not only was there muscle hypertrophy but also concomitant stimulation of muscle satellite cell proliferation and an increase of centrally nucleated muscle fibers, indicating regeneration. Although in one instance IGF-1 synthesis was mediated by a transgene expressed only by the muscle (Dobrowolny et al., 2005), it has not been established if the secreted IGF-1 acts on the muscle, the motor neuron, or both. At a minimum, these efforts illustrate the possibility of using trophic factor synthesis by

muscle (see section below) as a way of rescuing motor neurons. Added to this, a number of studies have shown that exercise is beneficial in ALS transgenic animals (Kirkinezos et al., 2003; Veldink et al., 2003), with exercise and IGF-1 exhibiting a synergistic effect resulting in an increase in median life span by 83 days (Kaspar et al., 2005). The mechanism underlying this synergism is not known, and the relevance for human ALS unclear, as no consensus has emerged from several efforts to evaluate a benefit of exercise in human disease (Drory et al., 2001; Pinto et al., 1999; Scarmeas et al., 2002).

A Model for Selective Vulnerability of Motor Neurons in ALS

Combining the lessons from multiple animal models used a determine pathogenic mechanisms in inherited ALS, the central insight is that although motor neuron degeneration and death is the primary cause of the progressive paralysis, toxicity is produced by damage developed not only within motor neurons, but also by other nonneuronal neighbors (Figure 3).

Onset and Early Phase

Damage directly within motor neurons is an important determinant of disease onset. The earliest event, prior to disease symptoms, is retraction of motor axons from their synapses onto muscles. During this phase, mutant SOD1 primarily acts directly within motor neurons, with aggregation of misfolded SOD1 damaging cellular machinery, especially mitochondria, so as to inhibit one or more normal functions. Anterograde axonal transport is also inhibited, as the consequence either of mitochondrial damage or of disorganization of axonal neurofilaments. SOD1 mutant action within the motor neuron is amplified by action within other cell types, especially the microglia which respond to the initial damage by amplifying it, thereby driving more rapid disease progression and spread.

Symptomatic

The symptomatic phase is characterized by a massive activation of microglia and astrocytes, in addition to continuing damage within motor neurons themselves. Misfolded SOD1 mutant within astrocytes, as well as their activation in response to neuronal damage, induces loss of the EAAT2 glutamate transporter, reducing rapid recovery of synaptic glutamate and driving an excitotoxic response that includes excessive influx of Ca^{2+} during repetitive firing of glutamate receptors on motor neurons. SOD1 mutant action directly within microglial and astrocytic cells may provoke reduced secretion of trophic molecules or increased release of toxic factors (e.g., nitric oxide, TNFα, or mutant SOD1 secreted or released by cell leakage or lysis). The latter molecules drive spread of toxicity to neighboring cells. Mutant SOD1 can itself activate microglia. In return, microglial cells, through secretion of toxic factors, harm their environment, including motor neurons.

End Stage

At end stage, denervation has produced paralysis accompanied by muscle atrophy. Activated microglia or astrocytes produce diffusible toxic products, including nitric oxide and/or TNFα, which accelerate disease spread. Loss of EAAT2 glutamate transporters from astrocytes drives repetitive firing of glutamate receptors and an ensuing excitotoxicity that is accompanied by excessive Ca^{2+} entry that further damages mitochondria and finally triggers a caspase-dependent cell suicide pathway within motor neurons.

Thus, selective sensitivity of motor neurons to ubiquitously expressed SOD1 mutants derives from the combination of risk factors shared by those neurons and their most intimately associated cellular neighbors. Seen this way, it is clear that the question is not whether the mitochondrial damage and ensuing oxidative damage hypothesis is right, any more than it is whether the hypotheses for glutamate-linked excitotoxicity, axonal transport clogging, loss of trophic support, or excessive release of toxic species are right. All these mechanisms (which have also been invoked in sporadic ALS) are right and all combine to underlie selective vulnerability. This is very good news for the design of therapies: intervention at any of these steps can, in principle, disrupt the toxic cascade between the motor neuron and its neighbors.

Growth Factor Therapies in ALS

Use of trophic factors has dominated recent efforts in clinical trials in ALS. All of these efforts were predicated on the hope that whatever the primary disease provoking insult, provision of increased levels of factors that could be trophic for motor neurons would be of benefit. These have lead to nearly uniform disappointment, providing little or no benefit for IGF-1 (Borasio et al., 1998; Lai et al., 1997) and no benefit for BDNF (BDNF Study Group, 1999) or CNTF (ALS CNTF Treatment Study Group, 1996). Aside from the absence of evidence that growth factors were limiting in ALS, the weakness of these approaches was insuring adequate delivery to the right cells. One method for eliminating blockage of drug delivery by the blood-brain barrier is direct, continuous infusion into either the brain (ICV) or spinal cord (intrathecal; Figure 4B). Continuous intrathecal administration of BDNF was found to be without benefit, but ICV infusion of IGF-1 extended survival in mice (Nagano et al., 2005a), and a phase I clinical trial using the same strategy showed a modest benefit to patients without adverse effects (Nagano et al., 2005b).

In animal models, efficacy in growth factor delivery has been achieved by either of two approaches: viral-mediated gene delivery to convert target cells into factories that secrete trophic factors and infusion of trophic molecules directly into the brain or spinal cord (Figure 4). For the first approach (Figure 4A), an IGF-1-encoding gene was inserted into adenoassociated virus (AAV), a small replication-defective virus that is episomally maintained within the nucleus of a host cell. Injection into muscle yielded viral expression within motor neurons for at least 1 year. Use of this strategy slowed disease progression in $hSOD1^{G93A}$ mice even when initiated after disease onset (Kaspar et al., 2003). Not surprisingly, success required the right trophic factor. The same AAV producing GDNF used in the same manner provided only a very modest benefit (Kaspar et al., 2003), consistent with prior efforts using GDNF encoded by adenovirus (Acsadi et al., 2002) or AAV (Wang et al., 2002) delivered intramuscularly.

With the potential that variants in the VEGF gene contribute to some examples of ALS (Table 1), delivery of an

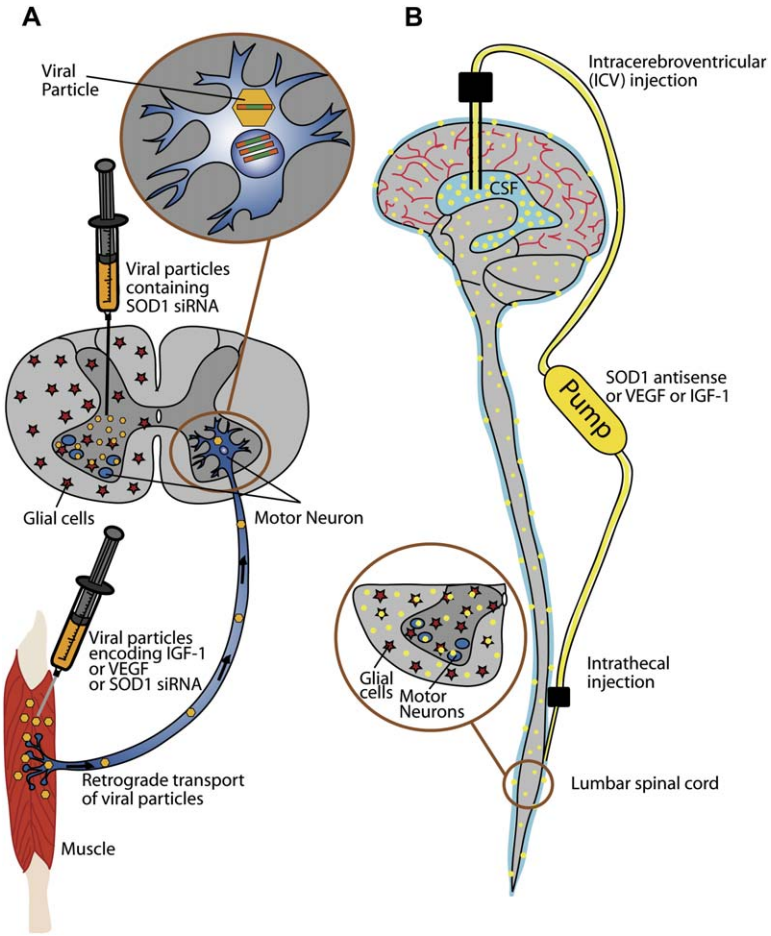

Figure 4. New Directions for Therapies in ALS Using Viral Delivery or Direct Infusion into the Brain or Spinal Cord

(A) Delivery by injection into muscle of viral particles (AAV) encoding trophic factors such as IGF-1 or VEGF. Viral particles are taken up at the synapse by the motor neuron terminal and retrogradely transported to the motor neuron cell body in the spinal cord. After translocation into the nucleus, the episomal viral genome is stably expressed for at least 1 year, converting motor neurons into factories for secretion of trophic factors. Trophic factors could also be synthesized directly in the muscle, increasing their support of the motor neuron terminal. This approach is also suitable for viral delivery of transcription-based siRNA approaches in which mutant SOD1 mRNAs (or specific cellular mRNAs) are targeted for degradation selectively within motor neurons. An alternative is for direct injection of viral particles into the spinal cord. This approach is more invasive but has the advantage of targeting not only the motor neurons but also nonneuronal neighboring cells of the motor neurons.

(B) Direct infusion into the spinal cord (intrathecal) or brain (intracerebroventricular, ICV) of trophic factors directly into the CSF yields widespread delivery throughout the brain and spinal cord. Alternatively, ICV infusion of antisense oligonucleotides can reduce mutant SOD1 by targeting intranuclear degradation of its mRNA both within the motor neurons and the surrounding microglial and astrocytic cells.

integrating lentivirus encoding *VEGF* (and pseudocoated so as to be retrogradely transported) extended survival of hSOD1^{G93A} mice (Azzouz et al., 2004). So too did continuous ICV infusion of recombinant VEGF protein into the CSF (Figure 4B): disease onset was delayed and survival extended by 22 days (Storkebaum et al., 2005). This ICV delivery of VEGF was especially effective in slowing forelimb paralysis, suggestive of a higher concentration of VEGF closer to the site of infusion. A modest benefit was seen even when VEGF treatment was initiated after symptomatic onset. Unresolved is which cells are targeted by this VEGF. Weekly intraperitoneal injection of VEGF also has been reported to slow disease in hSOD1^{G93A} mice (Zheng et al., 2004), raising the possibility that important target cells are outside the CNS, including those in the vasculature.

Gene Therapies in ALS

With recognition that the instances of ALS caused by dominant mutation in SOD1 derive from a toxic property of the mutant protein and that even complete absence of dismutase activity (by gene deletion) does not cause overt disease in mice (Reaume et al., 1996), strategies to limit the synthesis of the mutant gene product have become an increasingly realistic possibility. Two general strategies have been used: virally delivered, transcription-based RNA silencing (siRNA), and direct infusion of antisense oligonucleotides (Figure 4).

Three efforts using siRNA to catalyze degradation of the mRNA encoding SOD1 have proven successful in hSOD1^{G93A} mice. In the most comprehensive, a lentivirus encoding such an siRNA was injected into multiple muscles of very young mice (an age equivalent to injecting infants in humans). After retrograde transport to motor neuron cell bodies, reducing SOD1 mutant synthesis in motor neurons had a remarkable effect in slowing disease initiation (108 days) and extending overall survival by 99 days (Ralph et al., 2005). Despite sharply delayed disease onset through gene silencing within motor neurons but not other cells of the CNS, disease progression was not slowed at all, with animals reaching endstage ~25% *faster* in the treated animals (Ralph et al., 2005). Similar slowing of disease onset was seen by retrograde delivery of an siRNA-encoding AAV (Miller et al., 2005). Direct viral injection of a similar siRNA virus into the spinal cord (to target both motor neurons and their nonneuronal neighbors) delayed degeneration of motor neurons near the site of injection but it could not be determined whether this affected disease progression (Raoul et al., 2005).

In considering extension of such viral efforts to the human setting, substantial practical issues remain. Current viral vectors provide no mechanism for altering dosage or discontinuance of therapy and face significant challenges for reaching widespread areas of the CNS. An alternative approach that surmounts both of these is targeted mRNA degradation after direct ICV delivery of

synthetic antisense oligonucleotides into the CNS. Small (15–25) nucleotide sequences of DNA bind by Watson-Crick hybridization to a target mRNA in a sequence-specific manner. The mRNA in such a heteroduplex is a substrate for catalytic, intranuclear degradation by endogenous RNase H. This approach has been successfully used for lowering mutant SOD1 levels by ~50% throughout the brain and at all levels of the spinal cord of hSOD1^{G93A} rats. Initiated near disease onset, this approach successfully slowed progression after onset (Smith et al., 2006), a benchmark for a human therapy. Similar ICV administration to nonhuman primates demonstrated effective delivery to both the motor neurons and nonneuronal neighbors throughout the brains and spinal cords (Smith et al., 2006). These efforts establish that direct delivery of antisense oligonucleotides can be an effective, dosage-regulatable means of treating neurodegenerative diseases, including ALS. Added to this, direct infusion of siRNA duplexes, assuming issues of stability can be surmounted, add an additional therapeutic possibility, including the ability to target a mutant-encoding mRNA without affecting the wild-type mRNA (Xia et al., 2006).

Stem Cell Therapies in ALS

The selective, age-dependent killing of motor neurons has made ALS one of the poster diseases for cell replacement using stem cells. Replacement of human motor neurons, however, faces daunting challenges. The lower motor neurons have their cell bodies scattered throughout the length of the spinal cord, so that any cell replacement might require multiple spinal injections at all levels of the cord. Further, even if the developmental program can be achieved and issues of inhibition of axonal extension within the CNS are overcome, regrowth and correct targeting of meter-long axons will require at least a year (assuming the maximal rate of human axonal extension observed in early postnatal development).

These issues not withstanding, recent successes have overcome several of the important hurdles to the feasibility of a stem cell replacement strategy after acute motor neuron killing induced by Sindbis virus exposure (Deshpande et al., 2006; Harper et al., 2004). In a first series of experiments, mouse embryonic stem (ES) cells were differentiated into motor neuron precursors and were injected into rat spinal cords paralyzed by Sindbis virus exposure. A quarter (~3000 cells) of the in vitro differentiated, ES cell-derived motor neurons survived within the spinal cord and even a few of them extended axons into the ventral roots, but only when the rats were infused intrathecally with molecules known to alleviate myelin repulsion of axonal growth. However, neuromuscular junctions were not formed (Harper et al., 2004).

Altered differentiation conditions subsequently produced higher numbers of motor neurons derived from the ES cells and produced not only neuromuscular connections but relieved paralysis of Sindbis virus-exposed rats (Deshpande et al., 2006). A key improvement seems to be use of dibutyryl-cAMP as an anti-myelin repulsion factor and grafting of neural stem cells expressing glial-derived neurotrophic factor (GDNF) into the nerve. Transplantation of ES cells that had not been previously differentiated into motor neurons showed that the grafted motor neurons themselves, rather than other surviving derived stem cells, account for the improvement. Whether this strategy will be successful in a model of chronic motor neuron degeneration, such as ALS, remains to be determined.

Other strategies with hSOD1^{G93A} mice have used (1) human neural stem cells grafted into the spinal cord (Yan et al., 2006), (2) human umbilical cord blood cells transfused into the systemic circulation (Garbuzova-Davis et al., 2003), or (3) bone marrow transplant (Corti et al., 2004), and each has been reported to provide some extension in survival (Corti et al., 2004). One study reported an early protection of motor neurons which was not sustained at end stage (Corti et al., 2004).

An alternative strategy is the idea of stimulating the endogenous pool of stem cells for replacement of degenerating motor neurons. In hSOD1^{G93A} mice, an increase in neural progenitor cell proliferation, migration, and neurogenesis in the lumbar region of the spinal cord has been observed in response to motor neuron degeneration (Chi et al., 2006). Furthermore, the endogenous recruitment of neural progenitors (as determined by nestin staining) was initiated on the predominantly symptomatic side of asymmetrically paralyzed hSOD1^{G93A} rats (de Hemptinne et al., 2006). Whether endogenous neuroprogenitors could be used for generating new motor neurons is not yet established, although examination of the neural stem cell/neuroprogenitor niches of the forebrain has revealed alterations in hSOD1^{G93A} mice (Liu and Martin, 2006). It must also be recognized that any new neurons produced by such an approach will obligatorily express mutant protein and will also be at risk.

Finally, with the recognition of non-cell-autonomous contributions to motor neuron toxicity (Boillée et al., 2006; Clement et al., 2003), other stem cell strategies to replace nonneuronal precursors, including those engineered to produce trophic factors such as IGF-1, are now sensible approaches.

Acknowledgments

S.B. is a recipient of a Fondation pour la Recherche Medicale fellowship and an INSERM fellowship. C.V.V. is a recipient of a Development Grant from the Muscular Dystrophy Association and a fellowship from the PVA Spinal Cord Research Foundation. D.W.C. receives salary support from the Ludwig Institute for Cancer Research. This work has been supported by a grant from the NIH (NS27036) to D.W.C.

References

Abalkhail, H., Mitchell, J., Habgood, J., Orrell, R., and de Belleroche, J. (2003). A new familial amyotrophic lateral sclerosis locus on chromosome 16q12.1-16q12.2. Am. J. Hum. Genet. *73*, 383–389.

Ackerley, S., Thornhill, P., Grierson, A.J., Brownlees, J., Anderton, B.H., Leigh, P.N., Shaw, C.E., and Miller, C.C. (2003). Neurofilament heavy chain side arm phosphorylation regulates axonal transport of neurofilaments. J. Cell Biol. *161*, 489–495.

Acsadi, G., Anguelov, R.A., Yang, H., Toth, G., Thomas, R., Jani, A., Wang, Y., Ianakova, E., Mohammad, S., Lewis, R.A., and Shy, M.E. (2002). Increased survival and function of SOD1 mice after glial cell-derived neurotrophic factor gene therapy. Hum. Gene Ther. *13*, 1047–1059.

Afifi, A.K., Aleu, F.P., Goodgold, J., and MacKay, B. (1966). Ultrastructure of atrophic muscle in amyotrophic lateral sclerosis. Neurology *16*, 475–481.

Al-Chalabi, A., Andersen, P.M., Nilsson, P., Chioza, B., Andersson, J.L., Russ, C., Shaw, C.E., Powell, J.F., and Leigh, P.N. (1999). Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. Hum. Mol. Genet. *8*, 157–164.

ALS CNTF Treatment Study Group. (1996). A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. Neurology *46*, 1244–1249.

Azzouz, M., Hottinger, A., Paterna, J.C., Zurn, A.D., Aebischer, P., and Bueler, H. (2000). Increased motoneuron survival and improved neuromuscular function in transgenic ALS mice after intraspinal injection of an adeno-associated virus encoding Bcl-2. Hum. Mol. Genet. *9*, 803–811.

Azzouz, M., Ralph, G.S., Storkebaum, E., Walmsley, L.E., Mitrophanous, K.A., Kingsman, S.M., Carmeliet, P., and Mazarakis, N.D. (2004). VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature *429*, 413–417.

Basso, M., Massignan, T., Samengo, G., Cheroni, C., De Biasi, S., Salmona, M., Bendotti, C., and Bonetto, V. (2006). Insoluble mutant SOD1 is partly oligoubiquitinated in amyotrophic lateral sclerosis mice. J. Biol. Chem., in press. Published online August 30, 2006. 10.1074/jbc.M603489200.

Batulan, Z., Shinder, G.A., Minotti, S., He, B.P., Doroudchi, M.M., Nalbantoglu, J., Strong, M.J., and Durham, H.D. (2003). High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. J. Neurosci. *23*, 5789–5798.

BDNF Study Group (1999). A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). Neurology *52*, 1427–1433.

Beers, D.R., Henkel, J.S., Wang, J., Yen, A.A., Siklos, L., McKercher, S.R., and Appel, S.H. (2006). Wild-type microglia and Immune cells are neuroprotective in PU-1 knockout mice with familial ALS. Proc. Natl. Acad. Sci. USA, in press.

Bergemalm, D., Jonsson, P.A., Graffmo, K.S., Andersen, P.M., Brannstrom, T., Rehnmark, A., and Marklund, S.L. (2006). Overloading of stable and exclusion of unstable human superoxide dismutase-1 variants in mitochondria of murine amyotrophic lateral sclerosis models. J. Neurosci. *26*, 4147–4154.

Boillée, S., Yamanaka, K., Lobsiger, C.S., Copeland, N.G., Jenkins, N.A., Kassiotis, G., Kollias, G., and Cleveland, D.W. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. Science *312*, 1389–1392.

Borasio, G.D., Robberecht, W., Leigh, P.N., Emile, J., Guiloff, R.J., Jerusalem, F., Silani, V., Vos, P.E., Wokke, J.H., and Dobbins, T. (1998). A Placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I study group. Neurology *51*, 583–586.

Boston-Howes, W., Gibb, S.L., Williams, E.O., Pasinelli, P., Brown, R.H., Jr., and Trotti, D. (2006). Caspase-3 cleaves and inactivates the glutamate transporter EAAT2. J. Biol. Chem. *281*, 14076–14084.

Bowling, A.C., Schulz, J.B., Brown, R.H., Jr., and Beal, M.F. (1993). Superoxide dismutase activity, oxidative damage, and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. J. Neurochem. *61*, 2322–2325.

Browne, S.E., Bowling, A.C., Baik, M.J., Gurney, M., Brown, R.H., Jr., and Beal, M.F. (1998). Metabolic dysfunction in familial, but not sporadic, amyotrophic lateral sclerosis. J. Neurochem. *71*, 281–287.

Browne, S.E., Yang, L., DiMauro, J.P., Fuller, S.W., Licata, S.C., and Beal, M.F. (2006). Bioenergetic abnormalities in discrete cerebral motor pathways presage spinal cord pathology in the G93A SOD1 mouse model of ALS. Neurobiol. Dis. *22*, 599–610.

Bruening, W., Roy, J., Giasson, B., Figlewicz, D.A., Mushynski, W.E., and Durham, H.D. (1999). Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. J. Neurochem. *72*, 693–699.

Bruijn, L.I., Becher, M.W., Lee, M.K., Anderson, K.L., Jenkins, N.A., Copeland, N.G., Sisodia, S.S., Rothstein, J.D., Borchelt, D.R., Price, D.L., and Cleveland, D.W. (1997). ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron *18*, 327–338.

Bruijn, L.I., Houseweart, M.K., Kato, S., Anderson, K.A., Anderson, S.D., Ohama, E., Reaume, A.G., Scott, R.W., and Cleveland, D.W. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. Science *281*, 1851–1854.

Cardona, A.E., Pioro, E.P., Sasse, M.E., Kostenko, V., Cardona, S.M., Dijkstra, I.M., Huang, D., Kidd, G., Dombrowski, S., Dutta, R., et al. (2006). Control of microglial neurotoxicity by the fractalkine receptor. Nat. Neurosci. *9*, 917–924.

Chance, P.F., Rabin, B.A., Ryan, S.G., Ding, Y., Scavina, M., Crain, B., Griffin, J.W., and Cornblath, D.R. (1998). Linkage of the gene for an autosomal dominant form of juvenile amyotrophic lateral sclerosis to chromosome 9q34. Am. J. Hum. Genet. *62*, 633–640.

Charcot, J.M., and Joffroy, A. (1869). Deux cas d'atrophie musculaire progressive avec lesion de la substance grise et des faisceaux antero-lateraux de la moelle epiniere. Arch. Physiol. Neurol. Path. *2*, 744–754.

Chen, Y.Z., Bennett, C.L., Huynh, H.M., Blair, I.P., Puls, I., Irobi, J., Dierick, I., Abel, A., Kennerson, M.L., Rabin, B.A., et al. (2004). DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am. J. Hum. Genet. *74*, 1128–1135.

Chen, W., Saeed, M., Mao, H., Siddique, N., Dellefave, L., Hung, W.Y., Deng, H.X., Sufit, R.L., Heller, S.L., Haines, J.L., et al. (2006). Lack of association of VEGF promoter polymorphisms with sporadic ALS. Neurology *67*, 508–510.

Chi, L., Ke, Y., Luo, C., Li, B., Gozal, D., Kalyanaraman, B., and Liu, R. (2006). Motor neuron degeneration promotes neural progenitor cell proliferation, migration, and neurogenesis in the spinal cords of amyotrophic lateral sclerosis mice. Stem Cells *24*, 34–43. Published online August 11, 2005. 10.1634/stemcells.2005-0076.

Chung, M.J., and Suh, Y.L. (2002). Ultrastructural changes of mitochondria in the skeletal muscle of patients with amyotrophic lateral sclerosis. Ultrastruct. Pathol. *26*, 3–7.

Cifuentes-Diaz, C., Frugier, T., Tiziano, F.D., Lacene, E., Roblot, N., Joshi, V., Moreau, M.H., and Melki, J. (2001). Deletion of murine SMN exon 7 directed to skeletal muscle leads to severe muscular dystrophy. J. Cell Biol. *152*, 1107–1114.

Clark, L.N., Poorkaj, P., Wszolek, Z., Geschwind, D.H., Nasreddine, Z.S., Miller, B., Li, D., Payami, H., Awert, F., Markopoulou, K., et al. (1998). Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. Proc. Natl. Acad. Sci. USA *95*, 13103–13107.

Clement, A.M., Nguyen, M.D., Roberts, E.A., Garcia, M.L., Boillée, S., Rule, M., McMahon, A.P., Doucette, W., Siwek, D., Ferrante, R.J., et al. (2003). Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science *302*, 113–117.

Conforti, L., Tarlton, A., Mack, T.G., Mi, W., Buckmaster, E.A., Wagner, D., Perry, V.H., and Coleman, M.P. (2000). A Ufd2/D4Cole1e chimeric protein and overexpression of Rbp7 in the slow Wallerian degeneration (WldS) mouse. Proc. Natl. Acad. Sci. USA *97*, 11377–11382.

Corson, L.B., Strain, J.J., Culotta, V.C., and Cleveland, D.W. (1998). Chaperone-facilitated copper binding is a property common to several classes of familial amyotrophic lateral sclerosis-linked superoxide dismutase mutants. Proc. Natl. Acad. Sci. USA *95*, 6361–6366.

Corti, S., Locatelli, F., Donadoni, C., Guglieri, M., Papadimitriou, D., Strazzer, S., Del Bo, R., and Comi, G.P. (2004). Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. Brain *127*, 2518–2532.

Cote, F., Collard, J.F., and Julien, J.P. (1993). Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: A mouse model of amyotrophic lateral sclerosis. Cell *73*, 35–46.

Couillard-Despres, S., Zhu, Q., Wong, P.C., Price, D.L., Cleveland, D.W., and Julien, J.P. (1998). Protective effect of neurofilament heavy gene overexpression in motor neuron disease induced by mutant superoxide dismutase. Proc. Natl. Acad. Sci. USA *95*, 9626–9630.

Coulpier, M., Junier, M.P., Peschanski, M., and Dreyfus, P.A. (1996). Bcl-2 sensitivity differentiates two pathways for motoneuronal death in the wobbler mutant mouse. J. Neurosci. *16*, 5897–5904.

Cudkowicz, M.E., Shefner, J.M., Schoenfeld, D.A., Zhang, H., Andreasson, K.I., Rothstein, J.D., and Drachman, D.B. (2006). Trial of celecoxib in amyotrophic lateral sclerosis. Ann. Neurol. *60*, 22–31.

Dal Canto, M.C., and Gurney, M.E. (1994). Development of central nervous system pathology in a murine transgenic model of human amyotrophic lateral sclerosis. Am. J. Pathol. *145*, 1271–1279.

Damiano, M., Starkov, A.A., Petri, S., Kipiani, K., Kiaei, M., Mattiazzi, M., Flint Beal, M., and Manfredi, G. (2006). Neural mitochondrial Ca^{2+} capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. J. Neurochem. *96*, 1349–1361.

Darman, J., Backovic, S., Dike, S., Maragakis, N.J., Krishnan, C., Rothstein, J.D., Irani, D.N., and Kerr, D.A. (2004). Viral-induced spinal motor neuron death is non-cell-autonomous and involves glutamate excitotoxicity. J. Neurosci. *24*, 7566–7575.

de Hemptinne, I., Boucherie, C., Pochet, R., Bantubungi, K., Schiffmann, S.N., Maloteaux, J.M., and Hermans, E. (2006). Unilateral induction of progenitors in the spinal cord of hSOD1(G93A) transgenic rats correlates with an asymmetrical hind limb paralysis. Neurosci. Lett. *401*, 25–29.

De Jonghe, P., Mersivanova, I., Nelis, E., Del Favero, J., Martin, J.J., Van Broeckhoven, C., Evgrafov, O., and Timmerman, V. (2001). Further evidence that neurofilament light chain gene mutations can cause Charcot-Marie-Tooth disease type 2E. Ann. Neurol. *49*, 245–249.

Deng, H.X., Shi, Y., Furukawa, Y., Zhai, H., Fu, R., Liu, E., Gorrie, G.H., Khan, M.S., Hung, W.Y., Bigio, E.H., et al. (2006). Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. Proc. Natl. Acad. Sci. USA *103*, 7142–7147.

Deshpande, D.M., Kim, Y.S., Martinez, T., Carmen, J., Dike, S., Shats, I., Rubin, L.L., Drummond, J., Krishnan, C., Hoke, A., et al. (2006). Recovery from paralysis in adult rats using embryonic stem cells. Ann. Neurol. *60*, 32–44.

Doble, A. (1996). The pharmacology and mechanism of action of riluzole. Neurology *47*, S233–S241.

Dobrowolny, G., Giacinti, C., Pelosi, L., Nicoletti, C., Winn, N., Barberi, L., Molinaro, M., Rosenthal, N., and Musaro, A. (2005). Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. J. Cell Biol. *168*, 193–199.

Drachman, D.B., Frank, K., Dykes-Hoberg, M., Teismann, P., Almer, G., Przedborski, S., and Rothstein, J.D. (2002). Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. Ann. Neurol. *52*, 771–778.

Drory, V.E., Goltsman, E., Reznik, J.G., Mosek, A., and Korczyn, A.D. (2001). The value of muscle exercise in patients with amyotrophic lateral sclerosis. J. Neurol. Sci. *191*, 133–137.

Dupuis, L., di Scala, F., Rene, F., de Tapia, M., Oudart, H., Pradat, P.F., Meininger, V., and Loeffler, J.P. (2003). Up-regulation of mitochondrial uncoupling protein 3 reveals an early muscular metabolic defect in amyotrophic lateral sclerosis. FASEB J. *17*, 2091–2093.

Durham, H.D., Roy, J., Dong, L., and Figlewicz, D.A. (1997). Aggregation of mutant Cu/Zn superoxide dismutase proteins in a culture model of ALS. J. Neuropathol. Exp. Neurol. *56*, 523–530.

Echaniz-Laguna, A., Zoll, J., Ribera, F., Tranchant, C., Warter, J.M., Lonsdorfer, J., and Lampert, E. (2002). Mitochondrial respiratory chain function in skeletal muscle of ALS patients. Ann. Neurol. *52*, 623–627.

Ekegren, T., Grundstrom, E., Lindholm, D., and Aquilonius, S.M. (1999). Upregulation of Bax protein and increased DNA degradation in ALS spinal cord motor neurons. Acta Neurol. Scand. *100*, 317–321.

Elliott, J.L. (2001). Cytokine upregulation in a murine model of familial amyotrophic lateral sclerosis. Brain Res. Mol. Brain Res. *95*, 172–178.

Engelhardt, J.I., and Appel, S.H. (1990). IgG reactivity in the spinal cord and motor cortex in amyotrophic lateral sclerosis. Arch. Neurol. *47*, 1210–1216.

Evgrafov, O.V., Mersiyanova, I., Irobi, J., Van Den Bosch, L., Dierick, I., Leung, C.L., Schagina, O., Verpoorten, N., Van Impe, K., Fedotov, V., et al. (2004). Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat. Genet. *36*, 602–606.

Ferri, A., Sanes, J.R., Coleman, M.P., Cunningham, J.M., and Kato, A.C. (2003). Inhibiting axon degeneration and synapse loss attenuates apoptosis and disease progression in a mouse model of motoneuron disease. Curr. Biol. *13*, 669–673.

Figlewicz, D.A., Krizus, A., Martinoli, M.G., Meininger, V., Dib, M., Rouleau, G.A., and Julien, J.P. (1994). Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. Hum. Mol. Genet. *3*, 1757–1761.

Fischer, L.R., Culver, D.G., Tennant, P., Davis, A.A., Wang, M., Castellano-Sanchez, A., Khan, J., Polak, M.A., and Glass, J.D. (2004). Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. Exp. Neurol. *185*, 232–240.

Fischer, L.R., Culver, D.G., Davis, A.A., Tennant, P., Wang, M., Coleman, M., Asress, S., Adalbert, R., Alexander, G.M., and Glass, J.D. (2005). The WldS gene modestly prolongs survival in the SOD1G93A fALS mouse. Neurobiol. Dis. *19*, 293–300.

Fray, A.E., Ince, P.G., Banner, S.J., Milton, I.D., Usher, P.A., Cookson, M.R., and Shaw, P.J. (1998). The expression of the glial glutamate transporter protein EAAT2 in motor neuron disease: an immunohistochemical study. Eur. J. Neurosci. *10*, 2481–2489.

Frey, D., Schneider, C., Xu, L., Borg, J., Spooren, W., and Caroni, P. (2000). Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. J. Neurosci. *20*, 2534–2542.

Fukada, K., Zhang, F., Vien, A., Cashman, N.R., and Zhu, H. (2004). Mitochondrial proteomic analysis of a cell line model of familial amyotrophic lateral sclerosis. Mol. Cell Proteomics *3*, 1211–1223.

Furukawa, Y., Torres, A.S., and O'Halloran, T.V. (2004). Oxygen-induced maturation of SOD1: a key role for disulfide formation by the copper chaperone CCS. EMBO J. *23*, 2872–2881.

Furukawa, Y., Fu, R., Deng, H.X., Siddique, T., and O'Halloran, T.V. (2006). Disulfide cross-linked protein represents a significant fraction of ALS-associated Cu, Zn-superoxide dismutase aggregates in spinal cords of model mice. Proc. Natl. Acad. Sci. USA *103*, 7148–7153.

Ganel, R., Ho, T., Maragakis, N.J., Jackson, M., Steiner, J.P., and Rothstein, J.D. (2006). Selective up-regulation of the glial Na+-dependent glutamate transporter GLT1 by a neuroimmunophilin ligand results in neuroprotection. Neurobiol. Dis. *21*, 556–567. Published online November 7, 2005. 10.1016/j.nbd.2005.08.014.

Garbuzova-Davis, S., Willing, A.E., Zigova, T., Saporta, S., Justen, E.B., Lane, J.C., Hudson, J.E., Chen, N., Davis, C.D., and Sanberg, P.R. (2003). Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. J. Hematother. Stem Cell Res. *12*, 255–270.

Garcia, M.L., Singleton, A.B., Hernandez, D., Ward, C.M., Evey, C., Sapp, P.A., Hardy, J., Brown, R.H., Jr., and Cleveland, D.W. (2006). Mutations in neurofilament genes are not a significant primary cause of non-SOD1-mediated amyotrophic lateral sclerosis. Neurobiol. Dis. *21*, 102–109. Published online August 3, 2005. 10.1016/j.nbd.2005.06.016.

Glass, J.D., Brushart, T.M., George, E.B., and Griffin, J.W. (1993). Prolonged survival of transected nerve fibres in C57BL/Ola mice is an intrinsic characteristic of the axon. J. Neurocytol. *22*, 311–321.

Goldknopf, I.L., Sheta, E.A., Bryson, J., Folsom, B., Wilson, C., Duty, J., Yen, A.A., and Appel, S.H. (2006). Complement C3c and related protein biomarkers in amyotrophic lateral sclerosis and Parkinson's disease. Biochem. Biophys. Res. Commun. *342*, 1034–1039.

Goldstein, L.S., and Yang, Z. (2000). Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. Annu. Rev. Neurosci. *23*, 39–71.

Gong, Y.H., Parsadanian, A.S., Andreeva, A., Snider, W.D., and Elliott, J.L. (2000). Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. J. Neurosci. *20*, 660–665.

Gonzalez de Aguilar, J.L., Gordon, J.W., Rene, F., de Tapia, M., Lutz-Bucher, B., Gaiddon, C., and Loeffler, J.P. (2000). Alteration of the Bcl-x/Bax ratio in a transgenic mouse model of amyotrophic lateral sclerosis: evidence for the implication of the p53 signaling pathway. Neurobiol. Dis. *7*, 406–415.

Gordon, P.H., Moore, D.H., Gelinas, D.F., Qualls, C., Meister, M.E., Werner, J., Mendoza, M., Mass, J., Kushner, G., and Miller, R.G. (2004). Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis. Neurology *62*, 1845–1847.

Gould, T.W., Buss, R.R., Vinsant, S., Prevette, D., Sun, W., Knudson, C.M., Milligan, C.E., and Oppenheim, R.W. (2006). Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. J. Neurosci. *26*, 8774–8786.

Greenway, M.J., Andersen, P.M., Russ, C., Ennis, S., Cashman, S., Donaghy, C., Patterson, V., Swingler, R., Kieran, D., Prehn, J., et al. (2006). ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. Nat. Genet. *38*, 411–413.

Groeneveld, G.J., Veldink, J.H., van der Tweel, I., Kalmijn, S., Beijer, C., de Visser, M., Wokke, J.H., Franssen, H., and van den Berg, L.H. (2003). A randomized sequential trial of creatine in amyotrophic lateral sclerosis. Ann. Neurol. *53*, 437–445.

Gros-Louis, F., Laurent, S., Lopes, A.A., Khoris, J., Meininger, V., Camu, W., and Rouleau, G.A. (2003). Absence of mutations in the hypoxia response element of VEGF in ALS. Muscle Nerve *28*, 774–775.

Gros-Louis, F., Lariviere, R., Gowing, G., Laurent, S., Camu, W., Bouchard, J.P., Meininger, V., Rouleau, G.A., and Julien, J.P. (2004). A frameshift deletion in peripherin gene associated with amyotrophic lateral sclerosis. J. Biol. Chem. *279*, 45951–45956.

Gros-Louis, F., Gaspar, C., and Rouleau, G.A. (2006). Genetics of familial and sporadic amyotrophic lateral sclerosis. Biochim. Biophys. Acta, in press. Published online February 10, 2006. 10.1016/j.bbadis.2006.01.004.

Gurney, M.E., Pu, H., Chiu, A.Y., Dal Canto, M.C., Polchow, C.Y., Alexander, D.D., Caliendo, J., Hentati, A., Kwon, Y.W., Deng, H.X., et al. (1994). Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science *264*, 1772–1775.

Hadano, S., Hand, C.K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R.S., Miyamoto, N., Showguchi-Miyata, J., Okada, Y., Singaraja, R., et al. (2001). A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat. Genet. *29*, 166–173.

Hafezparast, M., Ahmad-Annuar, A., Hummerich, H., Shah, P., Ford, M., Baker, C., Bowen, S., Martin, J.E., and Fisher, E.M. (2003). Paradigms for the identification of new genes in motor neuron degeneration. Amyotroph. Lateral Scler. Other Motor Neuron Disord. *4*, 249–257.

Hall, E.D., Oostveen, J.A., and Gurney, M.E. (1998). Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS. Glia *23*, 249–256.

Hand, C.K., Khoris, J., Salachas, F., Gros-Louis, F., Lopes, A.A., Mayeux-Portas, V., Brewer, C.G., Brown, R.H., Jr., Meininger, V., Camu, W., and Rouleau, G.A. (2002). A novel locus for familial amyotrophic lateral sclerosis, on chromosome 18q. Am. J. Hum. Genet. *70*, 251–256.

Harper, J.M., Krishnan, C., Darman, J.S., Deshpande, D.M., Peck, S., Shats, I., Backovic, S., Rothstein, J.D., and Kerr, D.A. (2004). Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron-injured adult rats. Proc. Natl. Acad. Sci. USA *101*, 7123–7128.

Henkel, J.S., Engelhardt, J.I., Siklos, L., Simpson, E.P., Kim, S.H., Pan, T., Goodman, J.C., Siddique, T., Beers, D.R., and Appel, S.H. (2004). Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. Ann. Neurol. *55*, 221–235.

Henkel, J.S., Beers, D.R., Siklos, L., and Appel, S.H. (2006). The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. Mol. Cell Neurosci. *31*, 427–437. Published online December 5, 2005. 10.1016/j.mcn.2005.10.016.

Hensley, K., Fedynyshyn, J., Ferrell, S., Floyd, R.A., Gordon, B., Grammas, P., Hamdheydari, L., Mhatre, M., Mou, S., Pye, Q.N., et al. (2003). Message and protein-level elevation of tumor necrosis factor alpha (TNF alpha) and TNF alpha-modulating cytokines in spinal cords of the G93A-SOD1 mouse model for amyotrophic lateral sclerosis. Neurobiol. Dis. *14*, 74–80.

Hentati, A., Bejaoui, K., Pericak-Vance, M.A., Hentati, F., Speer, M.C., Hung, W.Y., Figlewicz, D.A., Haines, J., Rimmler, J., Ben Hamida, C., et al. (1994). Linkage of recessive familial amyotrophic lateral sclerosis to chromosome 2q33-q35. Nat. Genet. *7*, 425–428.

Hentati, A., Ouahchi, K., Pericak-Vance, M.A., Nijhawan, D., Ahmad, A., Yang, Y., Rimmler, J., Hung, W., Schlotter, B., Ahmed, A., et al. (1998). Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15-q22 markers. Neurogenetics *2*, 55–60.

Higgins, C.M., Jung, C., Ding, H., and Xu, Z. (2002). Mutant Cu, Zn superoxide dismutase that causes motoneuron degeneration is present in mitochondria in the CNS. J. Neurosci. *22*, RC215.

Higgins, C.M., Jung, C., and Xu, Z. (2003). ALS-associated mutant SOD1G93A causes mitochondrial vacuolation by expansion of the intermembrane space and by involvement of SOD1 aggregation and peroxisomes. BMC Neurosci. *4*, 16.

Hirano, A. (1991). Cytopathology of amyotrophic lateral sclerosis. Adv. Neurol. *56*, 91–101.

Hirano, A., Donnenfeld, H., Sasaki, S., and Nakano, I. (1984a). Fine structural observations of neurofilamentous changes in amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. *43*, 461–470.

Hirano, A., Nakano, I., Kurland, L.T., Mulder, D.W., Holley, P.W., and Saccomanno, G. (1984b). Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. *43*, 471–480.

Holzbaur, E.L., Howland, D.S., Weber, N., Wallace, K., She, Y., Kwak, S., Tchistiakova, L.A., Murphy, E., Hinson, J., Karim, R., et al. (2006). Myostatin inhibition slows muscle atrophy in rodent models of amyotrophic lateral sclerosis. Neurobiol. Dis. *23*, 697–707.

Hosler, B.A., Siddique, T., Sapp, P.C., Sailor, W., Huang, M.C., Hossain, A., Daube, J.R., Nance, M., Fan, C., Kaplan, J., et al. (2000). Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22. JAMA *284*, 1664–1669.

Houseweart, M.K., and Cleveland, D.W. (1999). Bcl-2 overexpression does not protect neurons from mutant neurofilament-mediated motor neuron degeneration. J. Neurosci. *19*, 6446–6456.

Howland, D.S., Liu, J., She, Y., Goad, B., Maragakis, N.J., Kim, B., Erickson, J., Kulik, J., DeVito, L., Psaltis, G., et al. (2002). Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc. Natl. Acad. Sci. USA *99*, 1604–1609.

Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., et al. (1998). Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature *393*, 702–705.

Ince, P.G., Tomkins, J., Slade, J.Y., Thatcher, N.M., and Shaw, P.J. (1998). Amyotrophic lateral sclerosis associated with genetic abnormalities in the gene encoding Cu/Zn superoxide dismutase: molecular pathology of five new cases, and comparison with previous reports and 73 sporadic cases of ALS. J. Neuropathol. Exp. Neurol. *57*, 895–904.

Jaarsma, D., Haasdijk, E.D., Grashorn, J.A., Hawkins, R., van Duijn, W., Verspaget, H.W., London, J., and Holstege, J.C. (2000). Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. Neurobiol. Dis. *7*, 623–643.

Jaarsma, D., Rognoni, F., van Duijn, W., Verspaget, H.W., Haasdijk, E.D., and Holstege, J.C. (2001). CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. Acta Neuropathol. (Berl.) *102*, 293–305.

Johnston, J.A., Dalton, M.J., Gurney, M.E., and Kopito, R.R. (2000). Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. USA *97*, 12571–12576.

Jonsson, P.A., Ernhill, K., Andersen, P.M., Bergemalm, D., Brannstrom, T., Gredal, O., Nilsson, P., and Marklund, S.L. (2004). Minute quantities of misfolded mutant superoxide dismutase-1 cause amyotrophic lateral sclerosis. Brain *127*, 73–88. Published online October 8, 2003. 10.1093/brain/awh005.

Jonsson, P.A., Graffmo, K.S., Andersen, P.M., Brannstrom, T., Lindberg, M., Oliveberg, M., and Marklund, S.L. (2006). Disulphide-reduced superoxide dismutase-1 in CNS of transgenic amyotrophic lateral sclerosis models. Brain *129*, 451–464. Published online December 5, 2005. 10.1093/brain/awh704.

Jordanova, A., De Jonghe, P., Boerkoel, C.F., Takashima, H., De Vriendt, E., Ceuterick, C., Martin, J.J., Butler, I.J., Mancias, P., Papasozomenos, S., et al. (2003). Mutations in the neurofilament light chain gene (NEFL) cause early onset severe Charcot-Marie-Tooth disease. Brain *126*, 590–597.

Jung, C., Higgins, C.M., and Xu, Z. (2002). A quantitative histochemical assay for activities of mitochondrial electron transport chain complexes in mouse spinal cord sections. J. Neurosci. Methods *114*, 165–172.

Kabashi, E., Agar, J.N., Taylor, D.M., Minotti, S., and Durham, H.D. (2004). Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. J. Neurochem. *89*, 1325–1335.

Kaspar, B.K., Llado, J., Sherkat, N., Rothstein, J.D., and Gage, F.H. (2003). Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. Science *301*, 839–842.

Kaspar, B.K., Frost, L.M., Christian, L., Umapathi, P., and Gage, F.H. (2005). Synergy of insulin-like growth factor-1 and exercise in amyotrophic lateral sclerosis. Ann. Neurol. *57*, 649–655.

Kawahara, Y., Ito, K., Sun, H., Aizawa, H., Kanazawa, I., and Kwak, S. (2004). Glutamate receptors: RNA editing and death of motor neurons. Nature *427*, 801.

Kawahara, Y., Sun, H., Ito, K., Hideyama, T., Aoki, M., Sobue, G., Tsuji, S., and Kwak, S. (2006). Underediting of GluR2 mRNA, a neuronal death inducing molecular change in sporadic ALS, does not occur in motor neurons in ALS1 or SBMA. Neurosci. Res. *54*, 11–14. Published online October 12, 2005. 10.1016/j.neures.2005.09.006.

Kawamata, T., Akiyama, H., Yamada, T., and McGeer, P.L. (1992). Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. Am. J. Pathol. *140*, 691–707.

Kawamura, Y., Dyck, P.J., Shimono, M., Okazaki, H., Tateishi, J., and Doi, H. (1981). Morphometric comparison of the vulnerability of peripheral motor and sensory neurons in amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. *40*, 667–675.

Kieran, D., Kalmar, B., Dick, J.R., Riddoch-Contreras, J., Burnstock, G., and Greensmith, L. (2004). Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat. Med. *10*, 402–405.

Kieran, D., Hafezparast, M., Bohnert, S., Dick, J.R., Martin, J., Schiavo, G., Fisher, E.M., and Greensmith, L. (2005). A mutation in dynein rescues axonal transport defects and extends the life span of ALS mice. J. Cell Biol. *169*, 561–567.

Kirby, J., Halligan, E., Baptista, M.J., Allen, S., Heath, P.R., Holden, H., Barber, S.C., Loynes, C.A., Wood-Allum, C.A., Lunec, J., and Shaw, P.J. (2005). Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS. Brain *128*, 1686–1706.

Kirkinezos, I.G., Hernandez, D., Bradley, W.G., and Moraes, C.T. (2003). Regular exercise is beneficial to a mouse model of amyotrophic lateral sclerosis. Ann. Neurol. *53*, 804–807.

Kirkinezos, I.G., Bacman, S.R., Hernandez, D., Oca-Cossio, J., Arias, L.J., Perez-Pinzon, M.A., Bradley, W.G., and Moraes, C.T. (2005). Cytochrome c association with the inner mitochondrial membrane is impaired in the CNS of G93A-SOD1 mice. J. Neurosci. *25*, 164–172.

Kislinger, T., Cox, B., Kannan, A., Chung, C., Hu, P., Ignatchenko, A., Scott, M.S., Gramolini, A.O., Morris, Q., Hallett, M.T., et al. (2006). Global survey of organ and organelle protein expression in mouse: Combined proteomic and transcriptomic profiling. Cell *125*, 173–186.

Klivenyi, P., Ferrante, R.J., Matthews, R.T., Bogdanov, M.B., Klein, A.M., Andreassen, O.A., Mueller, G., Wermer, M., Kaddurah-Daouk, R., and Beal, M.F. (1999). Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. Nat. Med. *5*, 347–350.

Kong, J., and Xu, Z. (1998). Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. J. Neurosci. *18*, 3241–3250.

Kong, J., and Xu, Z. (2000). Overexpression of neurofilament subunit NF-L and NF-H extends survival of a mouse model for amyotrophic lateral sclerosis. Neurosci. Lett. *281*, 72–74.

Kostic, V., Jackson-Lewis, V., de Bilbao, F., Dubois-Dauphin, M., and Przedborski, S. (1997). Bcl-2: prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. Science *277*, 559–562.

Kriz, J., Nguyen, M.D., and Julien, J.P. (2002). Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. Neurobiol. Dis. *10*, 268–278.

Kruman, I.I., Pedersen, W.A., Springer, J.E., and Mattson, M.P. (1999). ALS-linked Cu/Zn-SOD mutation increases vulnerability of motor neurons to excitotoxicity by a mechanism involving increased oxidative stress and perturbed calcium homeostasis. Exp. Neurol. *160*, 28–39.

Lai, E.C., Felice, K.J., Festoff, B.W., Gawel, M.J., Gelinas, D.F., Kratz, R., Murphy, M.F., Natter, H.M., Norris, F.H., and Rudnicki, S.A. (1997). Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. Neurology *49*, 1621–1630.

Lambrechts, D., Storkebaum, E., Morimoto, M., Del-Favero, J., Desmet, F., Marklund, S.L., Wyns, S., Thijs, V., Andersson, J., van Marion, I., et al. (2003). VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. Nat. Genet. *34*, 383–394.

Lariviere, R.C., Beaulieu, J.M., Nguyen, M.D., and Julien, J.P. (2003). Peripherin is not a contributing factor to motor neuron disease in a mouse model of amyotrophic lateral sclerosis caused by mutant superoxide dismutase. Neurobiol. Dis. *13*, 158–166.

Lee, M.K., Marszalek, J.R., and Cleveland, D.W. (1994). A mutant neurofilament subunit causes massive, selective motor neuron death: Implications for the pathogenesis of human motor neuron disease. Neuron *13*, 975–988.

Levine, J.B., Kong, J., Nadler, M., and Xu, Z. (1999). Astrocytes interact intimately with degenerating motor neurons in mouse amyotrophic lateral sclerosis (ALS). Glia *28*, 215–224.

Li, M., Ona, V.O., Guegan, C., Chen, M., Jackson-Lewis, V., Andrews, L.J., Olszewski, A.J., Stieg, P.E., Lee, J.P., Przedborski, S., and Friedlander, R.M. (2000). Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. Science *288*, 335–339.

Lino, M.M., Schneider, C., and Caroni, P. (2002). Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. J. Neurosci. *22*, 4825–4832.

Liu, Z., and Martin, L.J. (2006). The adult neural stem and progenitor cell niche is altered in amyotrophic lateral sclerosis mouse brain. J. Comp. Neurol. *497*, 468–488.

Liu, J., Lillo, C., Jonsson, P.A., Vande Velde, C., Ward, C.M., Miller, T.M., Subramaniam, J.R., Rothstein, J.D., Marklund, S., Andersen, P.M., et al. (2004). Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. Neuron *43*, 5–17.

Liu, J., Shinobu, L.A., Ward, C.M., Young, D., and Cleveland, D.W. (2005). Elevation of the Hsp70 chaperone does not effect toxicity in mouse models of familial amyotrophic lateral sclerosis. J. Neurochem. *93*, 875–882.

Lobsiger, C.S., Garcia, M.L., Ward, C.M., and Cleveland, D.W. (2005). Altered axonal architecture by removal of the heavily phosphorylated neurofilament tail domains strongly slows superoxide dismutase 1 mutant-mediated ALS. Proc. Natl. Acad. Sci. USA *102*, 10351–10356.

Lukas, T.J., Luo, W.W., Mao, H., Cole, N., and Siddique, T. (2006). Informatics-assisted protein profiling in a transgenic mouse model of amyotrophic lateral sclerosis. Mol. Cell Proteomics *5*, 1233–1244.

Mack, T.G., Reiner, M., Beirowski, B., Mi, W., Emanuelli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., et al. (2001). Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. Nat. Neurosci. *4*, 1199–1206.

Maragakis, N.J., Dykes-Hoberg, M., and Rothstein, J.D. (2004). Altered expression of the glutamate transporter EAAT2b in neurological disease. Ann. Neurol. *55*, 469–477.

Marin-Teva, J.L., Dusart, I., Colin, C., Gervais, A., van Rooijen, N., and Mallat, M. (2004). Microglia promote the death of developing Purkinje cells. Neuron *41*, 535–547.

Mattiazzi, M., D'Aurelio, M., Gajewski, C.D., Martushova, K., Kiaei, M., Beal, M.F., and Manfredi, G. (2002). Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. J. Biol. Chem. *277*, 29626–29633.

McGeer, P.L., McGeer, E.G., Kawamata, T., Yamada, T., and Akiyama, H. (1991). Reactions of the immune system in chronic degenerative neurological diseases. Can. J. Neurol. Sci. *18*, 376–379.

Mendonca, D.M., Chimelli, L., and Martinez, A.M. (2005). Quantitative evidence for neurofilament heavy subunit aggregation in motor neurons of spinal cords of patients with amyotrophic lateral sclerosis. Braz. J. Med. Biol. Res. *38*, 925–933.

Miller, T.M., Kaspar, B.K., Kops, G.J., Yamanaka, K., Christian, L.J., Gage, F.H., and Cleveland, D.W. (2005). Virus-delivered small RNA silencing sustains strength in amyotrophic lateral sclerosis. Ann. Neurol. *57*, 773–776.

Mitsumoto, H., Chad, D.A., and Pioro, E.P. (1998). Amyotrophic Lateral Sclerosis (Philadelphia: Davis).

Mizusawa, H., Matsumoto, S., Yen, S.H., Hirano, A., Rojas-Corona, R.R., and Donnenfeld, H. (1989). Focal accumulation of phosphorylated neurofilaments within anterior horn cell in familial amyotrophic lateral sclerosis. Acta Neuropathol. (Berl.) *79*, 37–43.

Mootha, V.K., Bunkenborg, J., Olsen, J.V., Hjerrild, M., Wisniewski, J.R., Stahl, E., Bolouri, M.S., Ray, H.N., Sihag, S., Kamal, M., et al. (2003). Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. Cell *115*, 629–640.

Mu, X., He, J., Anderson, D.W., Trojanowski, J.Q., and Springer, J.E. (1996). Altered expression of bcl-2 and bax mRNA in amyotrophic lateral sclerosis spinal cord motor neurons. Ann. Neurol. *40*, 379–386.

Nagai, M., Aoki, M., Miyoshi, I., Kato, M., Pasinelli, P., Kasai, N., Brown, R.H., Jr., and Itoyama, Y. (2001). Rats expressing human cytosolic copper-zinc superoxide dismutase transgenes with amyotrophic lateral sclerosis: associated mutations develop motor neuron disease. J. Neurosci. *21*, 9246–9254.

Nagano, I., Ilieva, H., Shiote, M., Murakami, T., Yokoyama, M., Shoji, M., and Abe, K. (2005a). Therapeutic benefit of intrathecal injection of insulin-like growth factor-1 in a mouse model of amyotrophic lateral sclerosis. J. Neurol. Sci. *235*, 61–68.

Nagano, I., Shiote, M., Murakami, T., Kamada, H., Hamakawa, Y., Matsubara, E., Yokoyama, M., Moritaz, K., Shoji, M., and Abe, K. (2005b). Beneficial effects of intrathecal IGF-1 administration in patients with amyotrophic lateral sclerosis. Neurol. Res. *27*, 768–772.

Nguyen, M.D., Lariviere, R.C., and Julien, J.P. (2001). Deregulation of Cdk5 in a mouse model of ALS: Toxicity alleviated by perikaryal neurofilament inclusions. Neuron *30*, 135–147.

Nguyen, M.D., D'Aigle, T., Gowing, G., Julien, J.P., and Rivest, S. (2004). Exacerbation of motor neuron disease by chronic stimulation of innate immunity in a mouse model of amyotrophic lateral sclerosis. J. Neurosci. *24*, 1340–1349.

Nishimura, A.L., Mitne-Neto, M., Silva, H.C., Oliveira, J.R., Vainzof, M., and Zatz, M. (2004a). A novel locus for late onset amyotrophic lateral sclerosis/motor neurone disease variant at 20q13. J. Med. Genet. *41*, 315–320.

Nishimura, A.L., Mitne-Neto, M., Silva, H.C., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J.R., Gillingwater, T., Webb, J., et al. (2004b). A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am. J. Hum. Genet. *75*, 822–831.

Niwa, J., Ishigaki, S., Hishikawa, N., Yamamoto, M., Doyu, M., Murata, S., Tanaka, K., Taniguchi, N., and Sobue, G. (2002). Dorfin ubiquitylates mutant SOD1 and prevents mutant SOD1-mediated neurotoxicity. J. Biol. Chem. *277*, 36793–36798.

Okado-Matsumoto, A., and Fridovich, I. (2002). Amyotrophic lateral sclerosis: a proposed mechanism. Proc. Natl. Acad. Sci. USA *99*, 9010–9014.

Oosthuyse, B., Moons, L., Storkebaum, E., Beck, H., Nuyens, D., Brusselmans, K., Van Dorpe, J., Hellings, P., Gorselink, M., Heymans, S., et al. (2001). Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat. Genet. *28*, 131–138.

Ostojic, J., Axelman, K., Lannfelt, L., and Froelich-Fabre, S. (2003). No evidence of linkage to chromosome 9q21-22 in a Swedish family with frontotemporal dementia and amyotrophic lateral sclerosis. Neurosci. Lett. *340*, 245–247.

Pardo, A.C., Wong, V., Benson, L.M., Dykes, M., Tanaka, K., Rothstein, J.D., and Maragakis, N.J. (2006). Loss of the astrocyte glutamate transporter GLT1 modifies disease in SOD1(G93A) mice. Exp. Neurol. *201*, 120–130.

Pasinelli, P., Houseweart, M.K., Brown, R.H., Jr., and Cleveland, D.W. (2000). Caspase-1 and -3 are sequentially activated in motor neuron death in Cu, Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. USA *97*, 13901–13906.

Pasinelli, P., Belford, M.E., Lennon, N., Bacskai, B.J., Hyman, B.T., Trotti, D., and Brown, R.H., Jr. (2004). Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. Neuron *43*, 19–30.

Patel, Y.J., Payne Smith, M.D., de Belleroche, J., and Latchman, D.S. (2005). Hsp27 and Hsp70 administered in combination have a potent protective effect against FALS-associated SOD1-mutant-induced cell death in mammalian neuronal cells. Brain Res. Mol. Brain Res. *134*, 256–274. Published online December 15, 2004. 10.1016/j.molbrainres.2004.10.028.

Pehar, M., Cassina, P., Vargas, M.R., Castellanos, R., Viera, L., Beckman, J.S., Estevez, A.G., and Barbeito, L. (2004). Astrocytic production of nerve growth factor in motor neuron apoptosis: implications for amyotrophic lateral sclerosis. J. Neurochem. *89*, 464–473.

Perry, V.H., Brown, M.C., Lunn, E.R., Tree, P., and Gordon, S. (1990). Evidence that very slow Wallerian degeneration in C57BL/Ola mice is an intrinsic property of the peripheral nerve. Eur. J. Neurosci. *2*, 802–808.

Pieri, M., Albo, F., Gaetti, C., Spalloni, A., Bengtson, C.P., Longone, P., Cavalcanti, S., and Zona, C. (2003). Altered excitability of motor neurons in a transgenic mouse model of familial amyotrophic lateral sclerosis. Neurosci. Lett. *351*, 153–156.

Pinto, A.C., Alves, M., Nogueira, A., Evangelista, T., Carvalho, J., Coelho, A., de Carvalho, M., and Sales-Luis, M.L. (1999). Can amyotrophic lateral sclerosis patients with respiratory insufficiency exercise? J. Neurol. Sci. *169*, 69–75.

Pontieri, F.E., Ricci, A., Pellicano, C., Benincasa, D., and Buttarelli, F.R. (2005). Minocycline in amyotrophic lateral sclerosis: a pilot study. Neurol. Sci. *26*, 285–287.

Pramatarova, A., Laganiere, J., Roussel, J., Brisebois, K., and Rouleau, G.A. (2001). Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. J. Neurosci. *21*, 3369–3374.

Puls, I., Jonnakuty, C., LaMonte, B.H., Holzbaur, E.L., Tokito, M., Mann, E., Floeter, M.K., Bidus, K., Drayna, D., Oh, S.J., et al. (2003). Mutant dynactin in motor neuron disease. Nat. Genet. *33*, 455–456.

Pun, S., Santos, A.F., Saxena, S., Xu, L., and Caroni, P. (2006). Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. Nat. Neurosci. *9*, 408–419.

Puttaparthi, K., and Elliott, J.L. (2005). Nonneuronal induction of immunoproteasome subunits in an ALS model: possible mediation by cytokines. Exp. Neurol. *196*, 441–451.

Ralph, G.S., Radcliffe, P.A., Day, D.M., Carthy, J.M., Leroux, M.A., Lee, D.C., Wong, L.F., Bilsland, L.G., Greensmith, L., Kingsman, S.M., et al. (2005). Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. Nat. Med. *11*, 429–433.

Raoul, C., Henderson, C.E., and Pettmann, B. (1999). Programmed cell death of embryonic motoneurons triggered through the Fas death receptor. J. Cell Biol. *147*, 1049–1062.

Raoul, C., Estevez, A.G., Nishimune, H., Cleveland, D.W., deLapeyriere, O., Henderson, C.E., Haase, G., and Pettmann, B. (2002). Motoneuron death triggered by a specific pathway downstream of Fas. potentiation by ALS-linked SOD1 mutations. Neuron *35*, 1067–1083.

Raoul, C., Abbas-Terki, T., Bensadoun, J.C., Guillot, S., Haase, G., Szulc, J., Henderson, C.E., and Aebischer, P. (2005). Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. Nat. Med. *11*, 423–428.

Raoul, C., Buhler, E., Sadeghi, C., Jacquier, A., Aebischer, P., Pettmann, B., Henderson, C.E., and Haase, G. (2006). Chronic activation in presymptomatic amyotrophic lateral sclerosis (ALS) mice of a feedback loop involving Fas, Daxx, and FasL. Proc. Natl. Acad. Sci. USA *103*, 6007–6012.

Reaume, A.G., Elliott, J.L., Hoffman, E.K., Kowall, N.W., Ferrante, R.J., Siwek, D.F., Wilcox, H.M., Flood, D.G., Beal, M.F., Brown, R.H., Jr., et al. (1996). Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. Nat. Genet. *13*, 43–47.

Reid, E., Kloos, M., Ashley-Koch, A., Hughes, L., Bevan, S., Svenson, I.K., Graham, F.L., Gaskell, P.C., Dearlove, A., Pericak-Vance, M.A., et al. (2002). A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10). Am. J. Hum. Genet. *71*, 1189–1194.

Ripps, M.E., Huntley, G.W., Hof, P.R., Morrison, J.H., and Gordon, J.W. (1995). Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. USA *92*, 689–693.

Robertson, J., Doroudchi, M.M., Nguyen, M.D., Durham, H.D., Strong, M.J., Shaw, G., Julien, J.P., and Mushynski, W.E. (2003). A neurotoxic peripherin splice variant in a mouse model of ALS. J. Cell Biol. *160*, 939–949.

Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J.P., Deng, H.X., et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature *362*, 59–62.

Rothstein, J.D., Martin, L.J., and Kuncl, R.W. (1992). Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. N. Engl. J. Med. *326*, 1464–1468.

Rothstein, J.D., Van Kammen, M., Levey, A.I., Martin, L.J., and Kuncl, R.W. (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann. Neurol. *38*, 73–84.

Rothstein, J.D., Dykes-Hoberg, M., Corson, L.B., Becker, M., Cleveland, D.W., Price, D.L., Culotta, V.C., and Wong, P.C. (1999). The copper chaperone CCS is abundant in neurons and astrocytes in human and rodent brain. J. Neurochem. *72*, 422–429.

Rothstein, J.D., Patel, S., Regan, M.R., Haenggeli, C., Huang, Y.H., Bergles, D.E., Jin, L., Dykes Hoberg, M., Vidensky, S., Chung, D.S., et al. (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature *433*, 73–77.

Rouleau, G.A., Clark, A.W., Rooke, K., Pramatarova, A., Krizus, A., Suchowersky, O., Julien, J.P., and Figlewicz, D. (1996). SOD1 mutation is associated with accumulation of neurofilaments in amyotrophic lateral sclerosis. Ann. Neurol. *39*, 128–131.

Ruddy, D.M., Parton, M.J., Al-Chalabi, A., Lewis, C.M., Vance, C., Smith, B.N., Leigh, P.N., Powell, J.F., Siddique, T., Meyjes, E.P., et al. (2003). Two families with familial amyotrophic lateral sclerosis are linked to a novel locus on chromosome 16q. Am. J. Hum. Genet. *73*, 390–396.

Samsam, M., Mi, W., Wessig, C., Zielasek, J., Toyka, K.V., Coleman, M.P., and Martini, R. (2003). The Wlds mutation delays robust loss of motor and sensory axons in a genetic model for myelin-related axonopathy. J. Neurosci. *23*, 2833–2839.

Sapp, P.C., Hosler, B.A., McKenna-Yasek, D., Chin, W., Gann, A., Genise, H., Gorenstein, J., Huang, M., Sailer, W., Scheffler, M., et al. (2003). Identification of two novel loci for dominantly inherited familial amyotrophic lateral sclerosis. Am. J. Hum. Genet. *73*, 397–403.

Sasaki, S., Komori, T., and Iwata, M. (2000). Excitatory amino acid transporter 1 and 2 immunoreactivity in the spinal cord in amyotrophic lateral sclerosis. Acta Neuropathol. (Berl.) *100*, 138–144.

Sasaki, S., Warita, H., Murakami, T., Abe, K., and Iwata, M. (2004). Ultrastructural study of mitochondria in the spinal cord of transgenic mice with a G93A mutant SOD1 gene. Acta Neuropathol. (Berl.) *107*, 461–474.

Sato, T., Nakanishi, T., Yamamoto, Y., Andersen, P.M., Ogawa, Y., Fukada, K., Zhou, Z., Aoike, F., Sugai, F., Nagano, S., et al. (2005). Rapid disease progression correlates with instability of mutant SOD1 in familial ALS. Neurology *65*, 1954–1957.

Scarmeas, N., Shih, T., Stern, Y., Ottman, R., and Rowland, L.P. (2002). Premorbid weight, body mass, and varsity athletics in ALS. Neurology *59*, 773–775.

Schaefer, A.M., Sanes, J.R., and Lichtman, J.W. (2005). A compensatory subpopulation of motor neurons in a mouse model of amyotrophic lateral sclerosis. J. Comp. Neurol. *490*, 209–219.

Schiffer, D., Cordera, S., Cavalla, P., and Migheli, A. (1996). Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. J. Neurol. Sci. *139*, 27–33.

Schmitt-John, T., Drepper, C., Mussmann, A., Hahn, P., Kuhlmann, M., Thiel, C., Hafner, M., Lengeling, A., Heimann, P., Jones, J.M., et al. (2005). Mutation of Vps54 causes motor neuron disease and defective spermiogenesis in the wobbler mouse. Nat. Genet. *37*, 1213–1215.

Shefner, J.M., Cudkowicz, M.E., Schoenfeld, D., Conrad, T., Taft, J., Chilton, M., Urbinelli, L., Qureshi, M., Zhang, H., Pestronk, A., et al. (2004). A clinical trial of creatine in ALS. Neurology *63*, 1656–1661.

Simpson, E.P., Henry, Y.K., Henkel, J.S., Smith, R.G., and Appel, S.H. (2004). Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. Neurology *62*, 1758–1765.

Smith, R.A., Miller, T.M., Yamanaka, K., Monia, B.P., Condon, T.P., Hung, G., Lobsiger, C.S., Ward, C.M., McAlonis-Downes, M., Wei, H., et al. (2006). Antisense oligonucleotide therapy for neurodegenerative disease. J. Clin. Invest. *116*, 2290–2296.

Sobue, G., Hashizume, Y., Yasuda, T., Mukai, E., Kumagai, T., Mitsuma, T., and Trojanowski, J.Q. (1990). Phosphorylated high molecular weight neurofilament protein in lower motor neurons in amyotrophic lateral sclerosis and other neurodegenerative diseases involving ventral horn cells. Acta Neuropathol. (Berl.) *79*, 402–408.

Solomon, J.N., Lewis, C.A., Ajami, B., Corbel, S.Y., Rossi, F.M., and Krieger, C. (2006). Origin and distribution of bone marrow-derived cells in the central nervous system in a mouse model of amyotrophic lateral sclerosis. Glia *53*, 744–753.

Spalloni, A., Albo, F., Ferrari, F., Mercuri, N., Bernardi, G., Zona, C., and Longone, P. (2004a). Cu/Zn-superoxide dismutase (GLY93→ALA) mutation alters AMPA receptor subunit expression and function and potentiates kainate-mediated toxicity in motor neurons in culture. Neurobiol. Dis. *15*, 340–350.

Spalloni, A., Pascucci, T., Albo, F., Ferrari, F., Puglisi-Allegra, S., Zona, C., Bernardi, G., and Longone, P. (2004b). Altered vulnerability to kainate excitotoxicity of transgenic-Cu/Zn SOD1 neurones. Neuroreport *15*, 2477–2480.

Storkebaum, E., Lambrechts, D., Dewerchin, M., Moreno-Murciano, M.P., Appelmans, S., Oh, H., Van Damme, P., Rutten, B., Man, W.Y., De Mol, M., et al. (2005). Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. Nat. Neurosci. *8*, 85–92.

Subramaniam, J.R., Lyons, W.E., Liu, J., Bartnikas, T.B., Rothstein, J., Price, D.L., Cleveland, D.W., Gitlin, J.D., and Wong, P.C. (2002). Mutant SOD1 causes motor neuron disease independent of copper chaperone-mediated copper loading. Nat. Neurosci. *5*, 301–307.

Takeuchi, H., Kobayashi, Y., Yoshihara, T., Niwa, J., Doyu, M., Ohtsuka, K., and Sobue, G. (2002). Hsp70 and Hsp40 improve neurite outgrowth and suppress intracytoplasmic aggregate formation in cultured neuronal cells expressing mutant SOD1. Brain Res. *949*, 11–22.

Takuma, H., Kwak, S., Yoshizawa, T., and Kanazawa, I. (1999). Reduction of GluR2 RNA editing, a molecular change that increases calcium influx through AMPA receptors, selective in the spinal ventral gray of patients with amyotrophic lateral sclerosis. Ann. Neurol. *46*, 806–815.

Terry, P.D., Kamel, F., Umbach, D.M., Lehman, T.A., Hu, H., Sandler, D.P., and Taylor, J.A. (2004). VEGF promoter haplotype and amyotrophic lateral sclerosis (ALS). J. Neurogenet. *18*, 429–434.

Teuchert, M., Fischer, D., Schwalenstoecker, B., Habisch, H.J., Bockers, T.M., and Ludolph, A.C. (2006). A dynein mutation attenuates motor neuron degeneration in SOD1(G93A) mice. Exp. Neurol. *198*, 271–274.

Tomkins, J., Usher, P., Slade, J.Y., Ince, P.G., Curtis, A., Bushby, K., and Shaw, P.J. (1998). Novel insertion in the KSP region of the neurofilament heavy gene in amyotrophic lateral sclerosis (ALS). Neuroreport *9*, 3967–3970.

Troost, D., Claessen, N., van den Oord, J.J., Swaab, D.F., and de Jong, J.M. (1993). Neuronophagia in the motor cortex in amyotrophic lateral sclerosis. Neuropathol. Appl. Neurobiol. *19*, 390–397.

Tummala, H., Jung, C., Tiwari, A., Higgins, C.M., Hayward, L.J., and Xu, Z. (2005). Inhibition of chaperone activity is a shared property of several Cu, Zn-superoxide dismutase mutants that cause amyotrophic lateral sclerosis. J. Biol. Chem. *280*, 17725–17731.

Turner, M.R., Cagnin, A., Turkheimer, F.E., Miller, C.C., Shaw, C.E., Brooks, D.J., Leigh, P.N., and Banati, R.B. (2004). Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. Neurobiol. Dis. *15*, 601–609.

Urushitani, M., Kurisu, J., Tsukita, K., and Takahashi, R. (2002). Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. J. Neurochem. *83*, 1030–1042.

Urushitani, M., Kurisu, J., Tateno, M., Hatakeyama, S., Nakayama, K., Kato, S., and Takahashi, R. (2004). CHIP promotes proteasomal degradation of familial ALS-linked mutant SOD1 by ubiquitinating Hsp/Hsc70. J. Neurochem. *90*, 231–244.

Urushitani, M., Sik, A., Sakurai, T., Nukina, N., Takahashi, R., and Julien, J.P. (2006). Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. Nat. Neurosci. *9*, 108–118. Published online December 20, 2005. 10.1038/nn1603.

Van Damme, P., Van Den Bosch, L., Van Houtte, E., Callewaert, G., and Robberecht, W. (2002). GluR2-dependent properties of AMPA receptors determine the selective vulnerability of motor neurons to excitotoxicity. J. Neurophysiol. *88*, 1279–1287.

Van Den Bosch, L., Tilkin, P., Lemmens, G., and Robberecht, W. (2002). Minocycline delays disease onset and mortality in a transgenic model of ALS. Neuroreport *13*, 1067–1070.

Van Den Bosch, L., Storkebaum, E., Vleminckx, V., Moons, L., Vanopdenbosch, L., Scheveneels, W., Carmeliet, P., and Robberecht, W. (2004). Effects of vascular endothelial growth factor (VEGF) on motor neuron degeneration. Neurobiol. Dis. *17*, 21–28.

Van Vught, P.W., Sutedja, N.A., Veldink, J.H., Koeleman, B.P., Groeneveld, G.J., Wijmenga, C., Uitdehaag, B.M., de Jong, J.M., Baas, F., Wokke, J.H., and Van den Berg, L.H. (2005). Lack of association between VEGF polymorphisms and ALS in a Dutch population. Neurology *65*, 1643–1645.

Vande Velde, C., Garcia, M.L., Yin, X., Trapp, B.D., and Cleveland, D.W. (2004). The neuroprotective factor Wlds does not attenuate mutant SOD1-mediated motor neuron disease. Neuromolecular Med. *5*, 193–203.

Vaughan, K.T., and Vallee, R.B. (1995). Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and p150Glued. J. Cell Biol. *131*, 1507–1516.

Veldink, J.H., Bar, P.R., Joosten, E.A., Otten, M., Wokke, J.H., and van den Berg, L.H. (2003). Sexual differences in onset of disease and response to exercise in a transgenic model of ALS. Neuromuscul. Disord. *13*, 737–743.

Verhoeven, K., De Jonghe, P., Coen, K., Verpoorten, N., Auer-Grumbach, M., Kwon, J.M., FitzPatrick, D., Schmedding, E., De Vriendt, E., Jacobs, A., et al. (2003). Mutations in the small GTP-ase late endosomal protein RAB7 cause Charcot-Marie-Tooth type 2B neuropathy. Am. J. Hum. Genet. *72*, 722–727.

Vielhaber, S., Winkler, K., Kirches, E., Kunz, D., Buchner, M., Feistner, H., Elger, C.E., Ludolph, A.C., Riepe, M.W., and Kunz, W.S. (1999). Visualization of defective mitochondrial function in skeletal muscle fibers of patients with sporadic amyotrophic lateral sclerosis. J. Neurol. Sci. *169*, 133–139.

Vijayvergiya, C., Beal, M.F., Buck, J., and Manfredi, G. (2005). Mutant superoxide dismutase 1 forms aggregates in the brain mitochondrial matrix of amyotrophic lateral sclerosis mice. J. Neurosci. *25*, 2463–2470.

Vleminckx, V., Van Damme, P., Goffin, K., Delye, H., Van Den Bosch, L., and Robberecht, W. (2002). Upregulation of HSP27 in a transgenic model of ALS. J. Neuropathol. Exp. Neurol. *61*, 968–974.

Vukosavic, S., Stefanis, L., Jackson-Lewis, V., Guegan, C., Romero, N., Chen, C., Dubois-Dauphin, M., and Przedborski, S. (2000). Delaying caspase activation by Bcl-2: a clue to disease retardation in a transgenic mouse model of amyotrophic lateral sclerosis. J. Neurosci. *20*, 9119–9125.

Wang, L.J., Lu, Y.Y., Muramatsu, S., Ikeguchi, K., Fujimoto, K., Okada, T., Mizukami, H., Matsushita, T., Hanazono, Y., Kume, A., et al. (2002). Neuroprotective effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. J. Neurosci. *22*, 6920–6928.

Wang, J., Slunt, H., Gonzales, V., Fromholt, D., Coonfield, M., Copeland, N.G., Jenkins, N.A., and Borchelt, D.R. (2003). Copper-binding-site-null SOD1 causes ALS in transgenic mice: aggregates of nonnative SOD1 delineate a common feature. Hum. Mol. Genet. *12*, 2753–2764.

Wang, J., Xu, G., Slunt, H.H., Gonzales, V., Coonfield, M., Fromholt, D., Copeland, N.G., Jenkins, N.A., and Borchelt, D.R. (2005). Coincident thresholds of mutant protein for paralytic disease and protein aggregation caused by restrictively expressed superoxide dismutase cDNA. Neurobiol. Dis. *20*, 943–952.

Watanabe, M., Dykes-Hoberg, M., Culotta, V.C., Price, D.L., Wong, P.C., and Rothstein, J.D. (2001). Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. Neurobiol. Dis. *8*, 933–941.

Waterman-Storer, C.M., Karki, S., and Holzbaur, E.L. (1995). The p150Glued component of the dynactin complex binds to both microtubules and the actin-related protein centractin (Arp-1). Proc. Natl. Acad. Sci. USA *92*, 1634–1638.

Wengenack, T.M., Holasek, S.S., Montano, C.M., Gregor, D., Curran, G.L., and Poduslo, J.F. (2004). Activation of programmed cell death markers in ventral horn motor neurons during early presymptomatic stages of amyotrophic lateral sclerosis in a transgenic mouse model. Brain Res. *1027*, 73–86.

West, M., Mhatre, M., Ceballos, A., Floyd, R.A., Grammas, P., Gabbita, S.P., Hamdheydari, L., Mai, T., Mou, S., Pye, Q.N., et al. (2004). The arachidonic acid 5-lipoxygenase inhibitor nordihydroguaiaretic acid inhibits tumor necrosis factor alpha activation of microglia and extends survival of G93A-SOD1 transgenic mice. J. Neurochem. *91*, 133–143.

Weydt, P., Yuen, E.C., Ransom, B.R., and Moller, T. (2004). Increased cytotoxic potential of microglia from ALS-transgenic mice. Glia *48*, 179–182.

Wiedemann, F.R., Manfredi, G., Mawrin, C., Beal, M.F., and Schon, E.A. (2002). Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients. J. Neurochem. *80*, 616–625.

Williamson, T.L., and Cleveland, D.W. (1999). Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. Nat. Neurosci. *2*, 50–56.

Williamson, T.L., Bruijn, L.I., Zhu, Q., Anderson, K.L., Anderson, S.D., Julien, J.P., and Cleveland, D.W. (1998). Absence of neurofilaments reduces the selective vulnerability of motor neurons and slows disease caused by a familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. Proc. Natl. Acad. Sci. USA *95*, 9631–9636.

Wong, P.C., Pardo, C.A., Borchelt, D.R., Lee, M.K., Copeland, N.G., Jenkins, N.A., Sisodia, S.S., Cleveland, D.W., and Price, D.L. (1995). An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. Neuron *14*, 1105–1116.

Wong, N.K., He, B.P., and Strong, M.J. (2000a). Characterization of neuronal intermediate filament protein expression in cervical spinal motor neurons in sporadic amyotrophic lateral sclerosis (ALS). J. Neuropathol. Exp. Neurol. *59*, 972–982.

Wong, P.C., Waggoner, D., Subramaniam, J.R., Tessarollo, L., Bartnikas, T.B., Culotta, V.C., Price, D.L., Rothstein, J., and Gitlin, J.D. (2000b). Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci. USA *97*, 2886–2891.

Xia, X., Zhou, H., Huang, Y., and Xu, Z. (2006). Allele-specific RNAi selectively silences mutant SOD1 and achieves significant therapeutic benefit in vivo. Neurobiol. Dis. *23*, 578–586.

Xu, Z., Cork, L.C., Griffin, J.W., and Cleveland, D.W. (1993). Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. Cell *73*, 23–33.

Yamada, K., Andrews, C., Chan, W.M., McKeown, C.A., Magli, A., de Berardinis, T., Loewenstein, A., Lazar, M., O'Keefe, M., Letson, R., et al. (2003). Heterozygous mutations of the kinesin KIF21A in congenital fibrosis of the extraocular muscles type 1 (CFEOM1). Nat. Genet. *35*, 318–321.

Yamanaka, K., Miller, T.M., McAlonis-Downes, M., Chun, S.J., and Cleveland, D.W. (2006). Progressive spinal axonal degeneration and slowness in ALS2-deficient mice. Ann. Neurol. *60*, 95–104.

Yan, J., Xu, L., Welsh, A.M., Chen, D., Hazel, T., Johe, K., and Koliatsos, V.E. (2006). Combined immunosuppressive agents or CD4 antibodies prolong survival of human neural stem cell grafts and improve disease outcomes in ALS transgenic mice. Stem Cells *24*, 1976–1985.

Yang, Y., Hentati, A., Deng, H.X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W.Y., Ouahchi, K., Yan, J., Azim, A.C., et al. (2001). The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat. Genet. *29*, 160–165.

Yasojima, K., Tourtellotte, W.W., McGeer, E.G., and McGeer, P.L. (2001). Marked increase in cyclooxygenase-2 in ALS spinal cord: implications for therapy. Neurology *57*, 952–956.

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I.P., Naylor, A., Bountra, C., Banati, R.R., and Anand, P. (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. BMC Neurol. *6*, 12.

Yoshida, S., Mulder, D.W., Kurland, L.T., Chu, C.P., and Okazaki, H. (1986). Follow-up study on amyotrophic lateral sclerosis in Rochester, Minn., 1925 through 1984. Neuroepidemiology *5*, 61–70.

Yoshihara, T., Ishigaki, S., Yamamoto, M., Liang, Y., Niwa, J., Takeuchi, H., Doyu, M., and Sobue, G. (2002). Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. J. Neurochem. *80*, 158–167.

Yrjanheikki, J., Tikka, T., Keinanen, R., Goldsteins, G., Chan, P.H., and Koistinaho, J. (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc. Natl. Acad. Sci. USA *96*, 13496–13500.

Zhang, B., Tu, P., Abtahian, F., Trojanowski, J.Q., and Lee, V.M. (1997). Neurofilaments and orthograde transport are reduced in ventral root axons of transgenic mice that express human SOD1 with a G93A mutation. J. Cell Biol. *139*, 1307–1315.

Zhao, C., Takita, J., Tanaka, Y., Setou, M., Nakagawa, T., Takeda, S., Yang, H.W., Terada, S., Nakata, T., Takei, Y., et al. (2001). Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bβ. Cell *105*, 587–597.

Zhao, W., Xie, W., Le, W., Beers, D.R., He, Y., Henkel, J.S., Simpson, E.P., Yen, A.A., Xiao, Q., and Appel, S.H. (2004). Activated microglia initiate motor neuron injury by a nitric oxide and glutamate-mediated mechanism. J. Neuropathol. Exp. Neurol. *63*, 964–977.

Zheng, C., Nennesmo, I., Fadeel, B., and Henter, J.I. (2004). Vascular endothelial growth factor prolongs survival in a transgenic mouse model of ALS. Ann. Neurol. *56*, 564–567.

Zhu, S., Stavrovskaya, I.G., Drozda, M., Kim, B.Y., Ona, V., Li, M., Sarang, S., Liu, A.S., Hartley, D.M., Wu du, C., et al. (2002). Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature *417*, 74–78.