# FROM CHARCOT TO LOU GEHRIG: DECIPHERING SELECTIVE MOTOR NEURON DEATH IN ALS

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Since its description by Charcot more than 130 years ago, the mechanism underlying the characteristic selective degeneration and death of motor neurons in amyotrophic lateral sclerosis has remained a mystery. Modern genetics has now identified mutations in two genes — *SOD1* and *ALS2* — as primary causes of the disease, and has implicated others as potential contributors. Insights into these abnormalities, together with errors in the handling of synaptic glutamate and the potential excitotoxic response that this alteration provokes, have provided leads for the development of new strategies to identify an as yet elusive remedy for this progressive, fatal disorder.

SPASTICITY
The persistent contraction of certain muscles, which causes stiffness and interferes with gait, movement or speech.

NEUROFILAMENT
A type of intermediate filament that is found only in neurons and serves as a cytoskeletal element that supports the axonal cytoplasm.

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The most common motor neuron disease in human adults is amyotrophic lateral sclerosis (ALS). The primary hallmark of ALS is the selective killing of motor neurons, which initiates a progressive paralysis in mid-life. Initially described[1] in 1869 by the famous French neurobiologist and physician Jean-Martin Charcot, ALS first became known as Charcot's sclerosis. ALS is now familiarly known in the United States as Lou Gehrig's disease, in honour of the great baseball player who developed the disease in the 1930s. ALS has claimed many other highly visibile victims, including the actor David Niven, the composer Dimitri Shostakovich, and the Chinese political leader, Mao Zedong. The Nobel-prize-winning astrophysicist Stephen Hawking suffers from an unusually slowly progressing form of the disease; this example highlights how selective the neuronal loss can be, as there is no cognitive impairment, despite the nearly complete paralysis of arms, legs and the muscles necessary for speech.

Generally fatal within 1–5 years of onset, ALS has a prevalence of 2–3 per 100,000 people. The risk increases by an order of magnitude after 60 years of age[2], and the disease is responsible for ~1 in every 800 deaths when a given population is followed longitudinally[2]. The causes of almost all occurrences of the disease remain unknown. In 90–95% of instances, there is no apparent genetic linkage (a form of the disease referred to as sporadic ALS), but in the remaining 5–10% of cases, the disease is inherited in a dominant manner (a form referred to as familial ALS). The typical age of onset for sporadic and most familial forms is between 50 and 60 years. The hallmark of both forms is progressive muscle weakness, atrophy and SPASTICITY, each of which reflects the degeneration and death of upper or lower motor neurons in the brain and spinal cord. It is less well recognized that at least 30% of small interneurons in the motor cortex and spinal cord also degenerate. Denervation of the respiratory muscles and diaphragm is generally the fatal event.

ALS is, in reality, a member of a group of heterogeneous disorders (TABLE 1). Over the years, many proposals for mechanisms that underlie the selective killing of upper and lower motor neurons have been put forward. The combination of genetic, pathological and biochemical post-mortem studies has fuelled four primary hypotheses for mechanisms that provoke or contribute to the disease: oxidative damage, an idea prompted by the discovery that mutations in SOD1 (the cytoplasmic copper/zinc superoxide dismutase) are a primary cause of ALS[3]; axonal strangulation from NEUROFILAMENTOUS disorganization, an idea supported by

GUANINE EXCHANGE FACTOR
A protein that facilitates the exchange of GDP for GTP in the nucleotide-binding pocket of a GTP-binding protein.

the abnormal accumulation of neurofilaments as a pathological feature of many cases of sporadic[4] and SOD1-mediated familial disease[5]; toxicity from intracellular aggregates and/or failure of protein folding or degradation, a common feature of ALS involving SOD1 mutations[6]; and repetitive motor neuron firing and subsequent excitotoxic death due to mishandling of glutamate, the neurotransmitter that acts on both upper and lower motor neurons. Here we provide a summary of what is now known about the involvement of each of these factors in sporadic and familial disease, and discuss the discovery of a second gene — a putative GUANINE EXCHANGE FACTOR (GEF) for a G protein that is so far unknown — mutations of which provoke an early-onset, recessive, slowly progressing form of ALS. We also review how molecular genetics in mice has highlighted the selective sensitivity of motor neurons to minor disturbances in vascular perfusion or trophic support, and examine what prospects lie ahead in terms of intervention for an affliction that has remained without an effective remedy since its description by Charcot.

## SOD1 mutations confer toxic properties

The first concrete insight into what provokes the selective killing of motor neurons in ALS was provided 8 years ago by a landmark discovery by a consortium of molecular geneticists and physicians, armed with modern genetic mapping methods and with DNAs from patients suffering from familial ALS. This consortium identified[3] mutations in SOD1 as the primary cause of ~15–20% of instances of familial ALS (FIG. 1), accounting, overall, for ~1–2% of cases of the disease. It is generally acknowledged that the clinical presentations of sporadic and familial ALS are indistinguishable, although there are detectable differences in pathology. For example, there is an expanded vulnerability of sensory neurons in the posterior column and spinocerebellar tract in SOD1-mutant-mediated ALS (at least in disease caused by the mutation $SOD1^{A4V}$ — the most common form in North America[5]). In addition, the age of onset of SOD1-mutant-mediated disease is perhaps a decade earlier than that of sporadic ALS, and the former starts more frequently in the lower extremities[7].

The discovery that mutations in the abundant, soluble, cytoplasmic enzyme SOD1 could underlie motor neuron death[3] was perplexing. It was not obvious at all how a mutation in SOD1 could be a primary cause of selective motor neuron degeneration. The known action of this 153-amino-acid, ubiquitously expressed, cytoplasmic homodimer (FIG. 1b) is to convert superoxide, which is produced primarily by errors of oxidative phosphorylation in mitochondria[8], to water and hydrogen peroxide. Catalysis by SOD1 is mediated in two asymmetric steps by an essential copper atom, which is alternately reduced and oxidized by superoxide (FIG. 2a). A distinct superoxide dismutase (manganese superoxide dismutase or SOD2) is resident in mitochondria, yet it is mutations in the cytoplasmic enzyme that yield ALS.

The conclusion that mutations in SOD1 cause ALS is now inescapable. More than 90 mutations (FIG. 1a) are known[9–11], and the list is certain to grow longer as more patients are analysed (a continually updated list can be found at the online database for SOD1/ALS genetic mutations — alsod.org). With one exception[12,13] ($SOD1^{D90A}$, see below), all of the mutations provoke dominantly inherited disease. Almost all examples are found in familial as opposed to sporadic ALS, and none of the mutations (except $SOD1^{D90A}$) has been found in individuals without ALS.

Despite an initial proposal[14] that mutations lie primarily along the dimer axis of SOD, it is now clear that they are scattered throughout the primary (FIG. 1a) and three-dimensional structure of the protein (FIG. 1b). None of the mutations eliminates the synthesis of a nearly full-length polypeptide, which contains binding

Table 1 | **Genetics of amyotrophic lateral sclerosis and related diseases**

| Disease | Inheritance | Linkage | Gene/protein | Onset/course | Features |
|---|---|---|---|---|---|
| ***Sporadic ALS*** | None | None | Largely unknown; NF-H (?), EAAT2 (?) | Adult | Includes >90% of incidences of ALS |
| ***Familial ALS*** | | | | | |
| ALS | Dominant | 21q22.1 | SOD1[3] | Adult | Comprises 20% of inherited cases; more than 90 mutants known, all but one of which is dominant |
| ALS with frontotemporal dementia | Dominant | 9q21–22 | ?[110] | Adult | Dementia |
| ALS with dementia/parkinsonism | Dominant | 17q21 | TAU | Adult | Dementia > parkinsonism, amyotrophy |
| ALS | X-linked | Xp11–Xq12 | ?[111] | Adult | |
| Juvenile type 1 | Recessive | 15q15–22 | ?[95] | Adolescence | Slowly progressing |
| Juvenile type 3 | Recessive | 2q33 | *ALS2*[96,97] | Adolescence | Slowly progressing; mutant gene product seems to be a guanine exchange factor |
| Juvenile | Dominant | 9q34 | ?[94] | Before 25 | Slowly progressing |

Numbers in superscript refer to corresponding references in the bibliography. ?, not known; ALS, amyotrophic lateral sclerosis; EAAT2, excitatory amino acid transporter 2; NF-H, neurofilament heavy polypeptide; SOD1, superoxide dismutase 1; TAU, microtubule-associated protein tau.

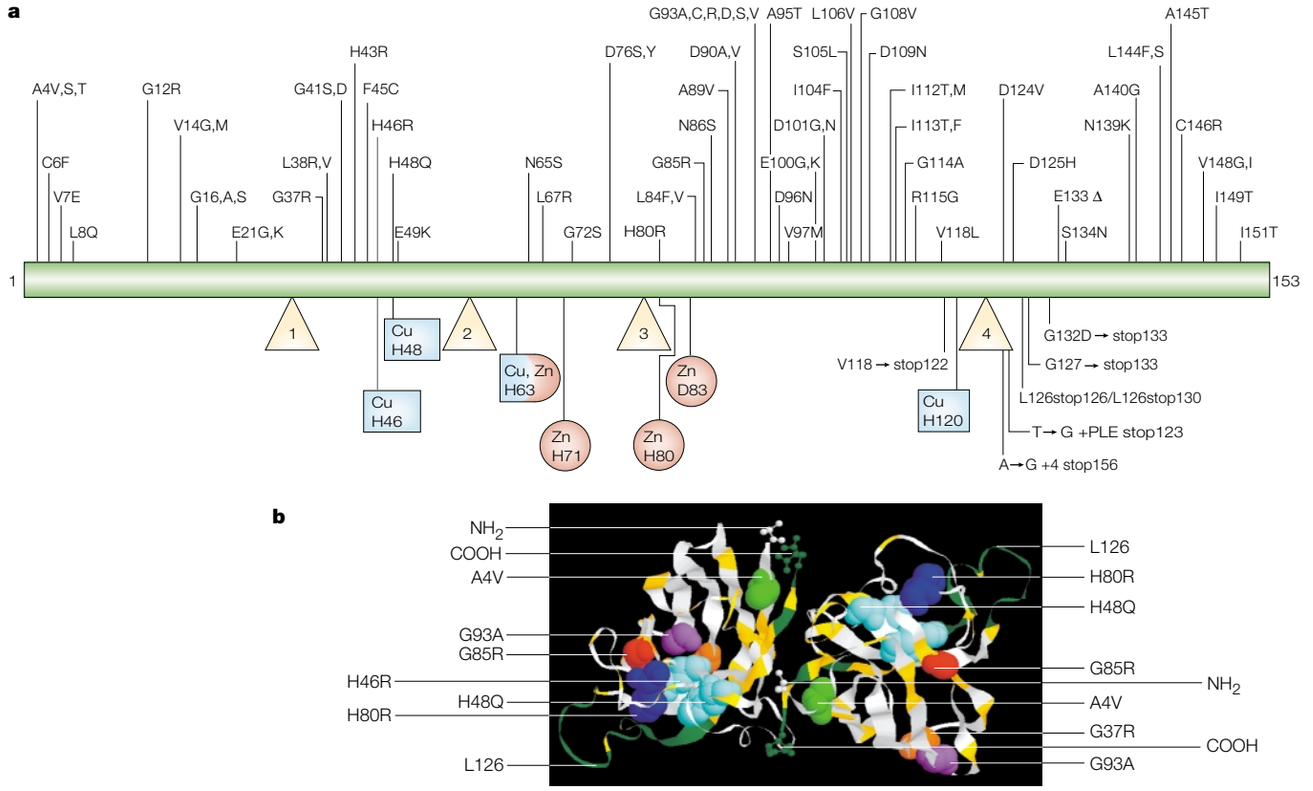


Figure 1 | **ALS-causing mutations lie throughout the SOD1 polypeptide. a** | Mutations in the 153-amino-acid superoxide dismutase 1 (SOD1) polypeptide that are known to cause amyotrophic lateral sclerosis (ALS). **b** | The positions of these mutations in the three-dimensional structure of crystallized human SOD1 (Protein Data Bank entry 1SPD) were highlighted using Rasmol (R. Sayle, Biomolecular Structures Group, GlaxoWellcome Research & Development). Mutations are scattered throughout the protein — at turns of the β-barrel, within β-sheets of the β-barrel, within the active site channel, at the dimer interface, and at copper-coordinating residues in the active site. Some mutated positions are marked with spacing-filling models of the amino-acid side chains. The positions of others are simply coloured yellow in the polypeptide chain. The 20 amino acids of the carboxyl terminus are truncated in several mutants and are coloured in green. The remaining polypeptide backbone is grey.

sites for a structural zinc and a catalytic copper, although three mutations alter one of the four residues that coordinate the copper, and at least seven splicing or FRAMESHIFT MUTATIONS truncate the final 20–30 amino acids. Although early measurements of SOD1 activity in patient blood indicated that a loss of enzymatic activity was central to the mechanism of disease[14,15], a transgenic mouse expressing the familial-ALS-linked mutant $SOD1^{G93A}$ developed progressive motor neuron disease despite markedly elevated SOD1 activity levels[16]. This finding was extended by three sets of mice that expressed different SOD1 mutants. In each of these cases, the mutations caused disease with elevated[17] or unchanged[18,19] SOD1 activity. These observations are in contrast to examples of mice in which SOD1 is completely deleted; these animals live uncompromised lifespans and do not develop overt motor neuron disease[20]. Furthermore, some mutants, such as $SOD1^{G37R}$, retain full specific activity[21]. In addition, neither the age of onset nor rapidity of progression of human disease correlate with dismutase activity levels[22,23]. The inevitable conclusion is that the mutants have acquired one or more toxic properties, irrespective of the amount of SOD1 activity that each of them retains.

FRAMESHIFT MUTATION
The addition or deletion of a nucleotide, which shifts the reading frame during translation such that the protein sequence from that point onwards is altered.

CASPASES
Cysteine proteases involved in apoptosis, which cleave at specific aspartate residues.

## Apoptotic neuronal death by caspase 3

Although the primary toxic property of familial-ALS-linked mutations in SOD1 remains unresolved, the final event in the death cascade has been established as activation of CASPASE type 3, one of the major cysteine–aspartate proteases responsible for the degradation of many key cellular constituents during apoptotic cell death. Initial evidence for such a role came from the finding that increasing the expression of the anti-apoptotic factor Bcl2 slowed disease onset and improved the survival of $SOD1^{G93A}$ mice by 3–4 weeks[24]. Activation of caspase 3 is a central feature in cell death mediated by mutant SOD1, appearing in motor neurons[25–27] and astrocytes[27] at the time of the earliest motor neuron death in all three of the best-studied mouse models. For one mutant ($SOD1^{G93A}$), the release of cytochrome *c* from mitochondria is followed[28] by activation of caspase 9, which might be the effector for the subsequent activation of caspase 3 and caspase 7.

A much earlier event in the mechanism of toxicity in SOD1 mutants is activation of caspase 1. This occurs months before neuronal death and before the appearance of the disease phenotype[25,27]. *In vitro*, this temporal cascade of caspase activation occurs within the same neuronal cells[27], although this has not been firmly established

in mice. Nevertheless, caspase inhibition by intrathecal administration of zVAD-fmk (*N*-benzylocarbonyl-Val-Ala-Asp-fluoromethylketone), a tetrapeptide pan-caspase inhibitor, prolongs the life of $SOD1^{G93A}$ mice by ~25% (REF. 26). So, a common toxic mechanism of mutant SOD1 is the sequential activation of at least two caspases that seem to act on a timescale much slower than that of apoptotic death during development.

### The Cu-mediated, oxidative damage hypothesis

Among the proposals to explain how more than 90 different mutations scattered throughout the SOD1 polypeptide can mediate the same neurodegenerative disease, the most obvious one is that less-tightly folded enzymes catalyse aberrant copper-mediated chemistry, owing to greater access of abnormal substrates to the active copper site. A slight modification of this idea is that mutants might handle the copper clumsily, frequently releasing it, allowing free copper to catalyse unwanted oxidative reactions. Consistent with these proposals, a common property of mutants, including those in which histidine residues that directly coordinate the copper atom are affected, is that they do bind some copper in at least one *in vivo* setting[29].

Among the first aberrant substrates to be proposed[30] was peroxynitrite ($^{-}$ONOO), which can form spontaneously from superoxide and nitric oxide, and, when used as a substrate, yields tyrosine nitration (FIG. 2c). Divergent evidence from several groups (primarily using immunocytochemistry) showed elevated levels of the predicted nitrotyrosine in SOD1-mediated disease in either mice[31–33] or humans[34]. However, evidence obtained by manipulating SOD1 activity levels in mice indicates that the peroxynitrite hypothesis is unlikely to have a central role. In mice that develop disease by expressing a mutant ($SOD1^{G85R}$) that confers only a ~10% increase in SOD1 activity over the wild-type SOD1, neither elimination nor chronic elevation (by about sixfold) of SOD1 activity affected disease onset, progression or pathology[6]. In another example, the expression of elevated levels of wild-type human SOD1 accelerated[35], rather than relieved, toxicity in mice carrying the $SOD1^{G93A}$ mutation, which confers significant activity by itself. The fact that toxicity cannot be ameliorated by increased SOD1 activity is inconsistent with damage arising from superoxide or any spontaneous reaction product of it (such as $^{-}$ONOO). If any such species were key to toxicity, elevation of the wild-type protein would be expected to diminish toxicity by lowering superoxide levels, whereas eliminating wild-type activity and forcing all catalysis through the mutant form would exacerbate toxicity. Neither of these predictions was found to be true.

A second proposed aberrant substrate is hydrogen peroxide — the normal end-product of the oxidized form of the enzyme ($SOD1–Cu^{2+}$). Use of peroxide as a substrate by the reduced $SOD1–Cu^{1+}$ form might produce the extraordinarily reactive hydroxyl radical (FIG. 2b), leading to a cascade of peroxidation. A two- to fourfold increase in the use of hydrogen peroxide by two mutants (relative to wild-type SOD1) has been reported *in vitro*[36]; however, this finding has been directly challenged on technical grounds[37]. Although products consistent with such a hypothesis have been reported in $SOD1^{G93A}$ mice[33,38], they have not been found at any stage of disease in others[32]. Furthermore, whether a higher level of peroxidation will arise from such a mechanism is not clear, as the increase in peroxide use by the mutant SOD1 could easily be counterbalanced by the decreased stability (and hence accumulated level) of many mutants[39].

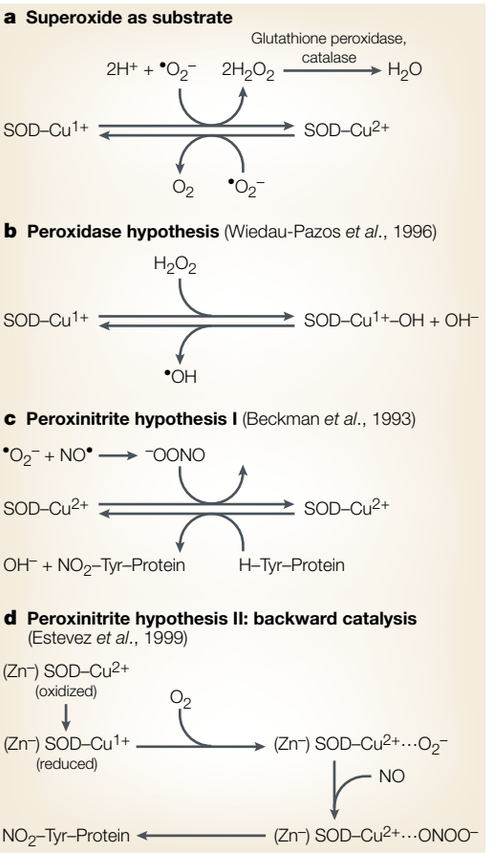


Figure 2 | **SOD1 chemistry. a** | Normal superoxide dismutase 1 (SOD1) chemistry: SOD1-mediated dismutation of superoxide in two asymmetric steps. **b**–**d** |Proposed toxic chemistries arising from the use of aberrant substrates. **b** | The formation of hydroxyl radicals by the use of hydrogen peroxide as an aberrant substrate by the reduced form of the enzyme (Wiedau-Pazos *et al.*[36]). **c**,**d** | Two models for protein nitration: the use of diffusible $^{-}$ONOO as an aberrant substrate (Beckman *et al.*[30]; **c**) and a zinc-depleted SOD1 mutant generating superoxide from $O_2$ (Estevez *et al.*[41]; **d**).

### Zn-deficient SOD1 as a generator of superoxide

A final specific proposal for aberrant chemistry mediated by SOD1 mutants arose from the *in vitro* finding that some mutants bind the structural zinc less well than wild-type SOD1 (REF. 40). Furthermore, the introduction of either wild-type or mutant SOD1, depleted of zinc, into primary motor neurons in culture was found to provoke rapid neuronal death in a manner that was dependent on neuronal nitric oxide synthase (nNOS), accompanied by elevated levels of protein-bound nitrotyrosine[41]. Toxicity required both zinc depletion

and bound copper. From these data, it was proposed that the reduction in bound zinc allowed a rapid reduction of mutant SOD1 to the $Cu^{1+}$ form by abundant intracellular reductants. The reduced SOD1 mutant would then run the normal catalytic step backwards, converting oxygen to superoxide. Finally, the superoxide so produced would combine in the enzymatic active site with freely diffusing nitric oxide, thereby producing peroxynitrite, which would promote intracellular damage, including protein nitration (FIG. 2d).

As attractive as this suggestion is, the acute toxicity of zinc-deficient wild-type or mutant SOD1 is likely to have little in common with the *in vivo* pathway of motor neuron death that arises from chronic expression of the mutants over a long period. After all, the wild-type protein is just as toxic as the mutants, and many SOD1 mutants do not show diminished zinc binding relative to wild-type SOD1 when produced *in vivo*[42,43]. Furthermore, limiting nitric oxide production by disrupting the nNOS gene does not affect disease onset or progression[44], and tyrosine nitration of proteins has not been detected in mice that develop disease from the $SOD1^{G37R}$ or $SOD1^{G85R}$ mutants[32,42], despite evidence for it in $SOD1^{G93A}$ mice[31]. Moreover, the expression of mutant, but not wild-type, SOD1 in primary motor neurons after gene microinjection induces neuronal death that is insensitive to NOS inhibitors and is not accompanied by increased nitrotyrosine[45], firmly indicating a SOD1-mutant-mediated toxicity that is independent of peroxynitrite.

### Testing the oxidative/catalytic hypothesis *in vivo*

In considering the proposals for aberrant substrates, increased levels of markers of oxidative damage have been reported in disease in some transgenic mice[31,33] but not in others[32,42], and in sporadic[31,46,47] but not in SOD1-mediated familial ALS[31,47]. A finding that seems to be consistent with oxidative damage is the early, transient appearance of damaged mitochondria, which vacuolate extensively within motor neurons[17,48] in mice expressing mutants that are highly active as dismutases. This is especially prominent at the onset of clinical features of the disease, but the vacuolation does not correlate with imminent neuronal death[49]. However, as similar vacuolation has been found with chronic, high-level expression of wild-type human SOD1 (REF. 35), but is not seen in disease caused by largely inactive mutants[18,19], it remains unclear how such mitochondrial dysfunction relates to a toxicity common to the many different ALS-linked SOD1 mutants. Indeed, this highlights a general danger in focusing too heavily on an outcome with a single mouse model, or even with several models if the mutants they express belong to a subset with similar chemical properties.

Amid such divergent findings, no conclusion can yet be drawn as to what degree of oxidative damage is central to the disease. However, the seminal discovery that copper acquisition by SOD1 in yeast requires a specific copper CHAPERONE for SOD1 (CCS)[50] has allowed testing of how the degree of copper loading affects toxicity. Both the human wild-type and mutant SOD1 subunits load copper *in vivo* through the action of a mammalian CCS[29,51]. In the absence of CCS, no copper loading onto mutant $SOD1^{G37R}$ was detected after *in vivo* labelling with radioactive copper, even though this mutant is fully active[21] and loads copper robustly in the presence of CCS (REF. 51 and J. R. Subramaniam *et al.*, unpublished observations). The striking finding is that sharply diminished copper loading has no effect on disease onset, progression or pathology in $SOD1^{G93A}$, $SOD1^{G37R}$ and $SOD1^{G85R}$ mice (J. R. Subramaniam *et al.*, unpublished observations), eliminating a contribution of CCS-dependent copper loading to mutant-generated toxicity.

Although this experiment offers powerful evidence against copper-mediated oxidative damage, there is one fly in the ointment. A residual amount of SOD1 activity (between 10% and 20%) is measured in an *in vitro* assay for SOD1 activity in tissue extracts from CCS-null, $SOD1^{G93A}$ and/or $SOD1^{G37R}$ mutant animals. It is not clear whether this reflects CCS-independent copper loading *in vivo*, or if it is an *in vitro* artefact that arises during tissue homogenization. Nevertheless, the complete insensitivity of toxicity to the absence of the CCS is not obviously consistent with an underlying oxidative mechanism. If CCS is absent, then most SOD1 will be copper free, and the amount of substrate available for the CCS-independent copper pathway will be chronically elevated by many fold. If such an alternative pathway loaded the copper into an aberrant site (as has been seen in some *in vitro* experiments[43]), and this CCS-independent copper loading of SOD1 were the toxic component, then the chronic increase in copper-free SOD1 in the absence of the CCS would be expected to drive increased toxicity through increased aberrant copper loading. This was found not to be the case. On the other hand, if an alternative pathway loaded the copper into the normal site on the enzyme, then a reduction in overall copper loading would be expected to lower toxicity correspondingly. Again, this outcome was found not to be correct.

Overall, despite the initial attractiveness of the oxidative hypothesis, three lines of evidence continue to offer strong evidence against the primary toxicity of SOD1 mutants arising from aberrant oxidative chemistry: first, the insensitivity of toxicity to the level of SOD1 activity; second, undiminished toxicity despite lower copper loading *in vivo* in mice (J. R. Subramaniam *et al.*, unpublished observations); and third, the paucity of evidence for increased markers of oxidative damage in SOD1-mediated human disease[47,52] and in some examples of disease in mice[42].

### Are intracellular aggregates of SOD1 toxic?

Studies of several neurodegenerative diseases (Alzheimer's disease, prion diseases, and polyglutamine diseases such as Huntington's disease) have revealed a common feature — protein aggregates. This commonality has fuelled a long-standing debate over whether these aggregates are central to disease pathogenesis, harmless by-products, or potentially beneficial through the sequestration of aberrant products. For ALS, a hypothesis of toxicity from protein aggregation initially

CHAPERONE
A protein that mediates the folding or assembly of another polypeptide, but does not form part of the completed structure, or participate in its biological function.

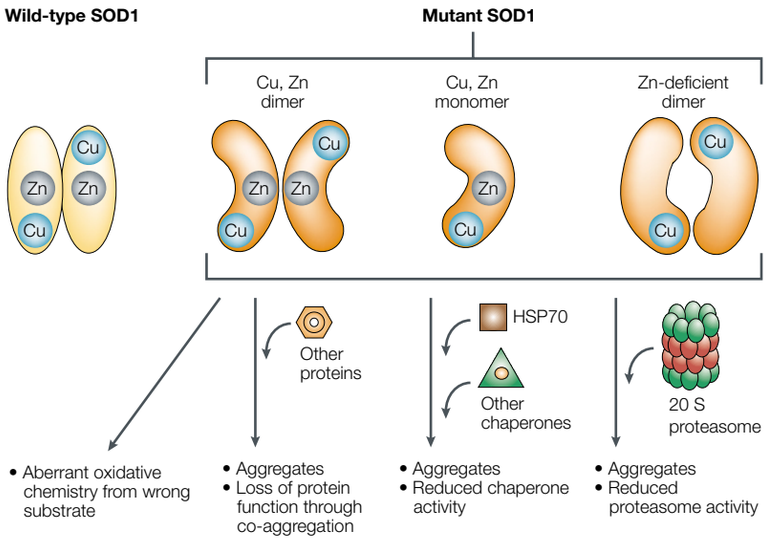


Figure 3 | **Models for SOD1-mediated toxicity linked to altered conformation and/or aggregation of mutant SOD1 subunits.** Toxicity from aberrant oxidative chemistry of misfolded or aggregated subunits that admit inappropriate substrates to the catalytic copper. Toxicity might occur by the following routes: first, by a loss of essential components through co-precipitation with mutant superoxide dismutase 1 (SOD1) in diffuse or focal aggregates[6,58]; second, by failure to fold/re-fold essential protein components after a reduction in overall protein-folding chaperone activity by the chronic folding or refolding of the abundant mutant SOD1 (REFS 57,109); or third, by inhibition of proteasome degradation by misfolded SOD1 mutants[53]. HSP70, heat-shock protein (70 kDa).

arose from the discovery of prominent, intracellular, cytoplasmic inclusions in motor neurons, and, in some cases, within the astrocytes surrounding them, in each of the prominent mouse models of SOD1-mediated ALS[6]. These aggregates develop before the onset of clinical symptoms. In some cases (for example, in SOD1$^{G85R}$ mice), they represent the first pathological sign of disease, and increase markedly in abundance during disease progression[6].

Intensely immunoreactive with antibodies to SOD1, the aggregates are of at least two classes. The first comprises those identifiable by conventional histological stains, which most frequently reveal intense, concentrated SOD1 immunoreactivity throughout the inclusion or, less frequently, only at the periphery of it. The second class includes perikaryal deposits that stain more diffusely. Aggregation, or at least the presence of misfolded subunits, therefore seems to be a characteristic of the SOD1 mutants. Moreover, misfolded SOD1 aggregates that cannot be readily dissociated, even with strong detergents and reducing agents, can be detected biochemically[53] in mice that will develop disease months later as a result of expressing SOD1$^{G93A}$.

Aspects of toxicity can arise either through aberrant chemistry, mediated by the misfolded aggregated mutants, or through loss or sequestration of essential cellular components (FIG. 3); for example, by saturating the protein-folding chaperones and/or the protein-degradation machinery. Consistent with the latter, the aggregates are intensely immunoreactive with antibodies to ubiquitin, a feature common not only to all instances of disease in mice[6], but also to many human examples[54–56]. Partial inhibition of the PROTEASOME is sufficient to provoke large aggregates in non-neuronal cells that express SOD1 mutants, leading to the proposal that proteasome activity could be limiting by combating such aggregates and, moreover, that undue proteasomal attention to aberrantly folded forms of SOD1 could compromise the removal of even more important components[53].

In this context, microinjection of genes encoding mutant, but not wild-type, SOD1 has yielded what might be the best *in vitro* model of ALS[57,58]. The forced expression of SOD1 mutants provokes mutant-dependent aggregates selectively in motor neurons, but not in sensory or hippocampal neurons, followed by cell death. Both aggregates and acute toxicity can be ameliorated by simultaneously elevating the level of the protein-folding chaperone HSP70 (heat-shock protein, 70 kDa). Overall chaperone activity (that is, the ability to prevent heat denaturation of added tester proteins) is modestly diminished (~25%) in spinal cord extracts from the SOD1$^{G93A}$ mice compared with normal extracts[57]. Combined with the absence of a robust shock response in many neurons, perhaps the most attractive model emerges for toxicity arising from mutant-mediated protein misfolding. The mutant SOD1 chronically ties up chaperones that are needed for catalysing the folding/refolding of more important substrates, whereas ubiquitin-mediated protein degradation by the proteasome is choked by these same aggregates. As ubiquitin-containing aggregates are a frequent feature of sporadic disease[54–56], this could link the mechanisms of familial and sporadic ALS, although aggregates containing SOD1 are not a characteristic feature of the sporadic form of the disorder.

### Are motor neurons direct targets for toxicity?

Independent of the mechanism of toxicity, one very basic question has not been resolved: does the toxicity that leads to motor neuron degeneration and death arise from damage directly to cells that express the mutant SOD1? This question of whether toxicity is CELL AUTONOMOUS initially arose from the ubiquitous expression of SOD1, but was heightened by pathology arising in astrocytes very early in disease[19], especially from mutant SOD1$^{G85R}$. The essential nature of glial cells in supporting motor neurons with trophic factors, and/or through the maintenance of glutamate homeostasis by an active uptake of excess glutamate through glutamate transporters[59], makes plausible a glial role (even a primary one) in SOD1-mediated toxicity. The selective expression of SOD1$^{G85R}$ in glia provokes some glial cell pathology, but not motor neuron death[60], indicating that only part of the toxicity can arise from glia. For motor neurons, the known influence on SOD1-mutant-mediated toxicity of some neuron-specific components, such as axonal neurofilament content[61,62] (see below), clearly supports a direct role of mutant-mediated toxicity within neurons; however, this has yet to be confirmed. In fact, a direct test of this idea, by forcing mutant SOD1$^{G37R}$ expression preferentially in neurons (with a neurofilament promoter), failed to produce neuronal degeneration[63], although the low level of expression in this case precludes a clear interpretation.

PROTEASOME
A protein complex responsible for degrading intracellular proteins that have been tagged for destruction by the addition of ubiquitin.

CELL AUTONOMOUS
A genetic trait in which only genotypically mutant cells show the mutant phenotype. In contrast, a cell non-autonomous trait is one in which genotypically mutant cells cause other cells (regardless of their genotype) to show a mutant phenotype.

Table 2 | **Evaluation of putative therapeutics in mice and humans**

| Compound category | Mutant SOD1 therapy | ALS clinical trials | Clinical trial comments |
|---|---|---|---|
| Antioxidant | SOD1[6,112], catalase[113], desmethylsegeline, lipoic acid[114], ginseng root[115], ginko biloba, *N*-acetylcysteine[116], MnSOD[117], nNOS null[44], general NOS inhibitor[118,119], ascorbate/trientine[120], vitamin E[121], carboxyfullerenes[122], glutathione peroxidase | Vitamin E[123], coenzyme Q10, *N*-acetylcysteine, selegeline, topiramate | None efficacious; one superoxide dismutase and catalase mimetic yielded a 10% extension of life span[112] |
| Metal ion regulation | Trientine[120], D-penicillamine[124], CCS deletion | | |
| Anti-inflammatory, immunomodulatory | cyclosporin[125], aspirin[126], indomethecin, FK506, celecoxib[127,*] | Gangliosides, interferon, cyclophosphamide, plasmaphoresis, intravenous γ-globulin, levamisole, celcoxibe, total lymphoid irradiation[128–135] | |
| Anti-apoptotics | zVAD-fmk[26,136,*], p53[137], p35, Bcl2[24], caspase 1[136], Bax, PARP[114] | | Broad-spectrum caspase inhibitor zVAD-fmK extended lifespan by 25%[26] |
| Structural proteins | NF-H[62,69,*], NF-L[69,*], NF-L deletion[61] | | Increasing NF-H represents the most effective extenison of lifespan — by up to 6 months[62] |
| Protein aggregation | HSP70[‡] | | |
| Calcium regulation | Calbindin | Verapamil, nimodepine[138,139] | None efficacious |
| Anti-glutamate | Riluzole[121], topiramate, naaladase inhibitors[140],GYKI-52466*[‡], EAAT2[141,*] | Riluzole, lamotrigine, dextromethorphan, LY300164 | Riluzole had a modest effect at increasing survival in two independent trials |
| Energy metabolism | Creatine[107,*], coenzyme Q10 | Creatine, BCAAs[142] | Initial BCAA trial positive; follow-up trials could not replicate |
| Trophic factors | BDNF, GDNF[143], sonic hedgehog, BMP7[144], cardiotropin 1[145], HGF[146] | Ciliary neurotrophic factor, BDNF, GDNF, TRH, xaliproden, IGF[101,103,104,147–149] | IGF modestly positive in US trials; failure to see efficacy in a European trial |
| Others | Gabapentin[121], genistein[150], GPI-1046, Jax kinase inhibitor[118,*] | Amantidine, ceftriaxone, octacosanol, tilorone, guanidine, isoprinosine[151–155] | |
| Stem cells | Umbilical cord blood[156], C17.2 (fetal neural)[157] | | |

Numbers in superscript refer to corresponding references in the bibliography. *Drug/treatment that increased survival by more than 20% of lifespan. ‡Authors' unpublished observations. ALS, amyotrophic lateral scerosis; BCAA, branched-chain amino acid; BDNF, brain-derived neurotrophic factor; BMP7, brain morphogenetic protein 7; CCS, copper chaperone for SOD1; EAAT2, excitatory amino acid transporter 2; GDNF, glial-derived neurotrophic factor; HGF, hepatocyte growth factor; HSP70, heat-shock protein (70 kDa); IGF, insulin-like growth factor; MnSOD, manganese superoxide dismutase (SOD2); NF-H, neurofilament heavy polypeptide; NF-L, neurofilament light polypeptide; nNOS, neuronal nitric oxide synthase; p35, regulatory subunit of cyclin-dependent kinase 5; p53, neuronal activator of cyclin-dependent kinase 5; PARP, poly(ADP-ribose) polymerase; SOD1, superoxide dismutase 1; TRH, thyrotropin-releasing hormone; zVAD-fmk, *N*-benzylocarbonyl-Val-Ala-Asp-fluoromethylketone.

## Neurofilaments as contributors and risk factors

From early reports of both sporadic[4,64] and familial[5] ALS, neurofilament accumulations in the cell bodies (FIG. 4a) and proximal axons (FIG. 4b) of motor neurons have been established as hallmarks of the disease. The further demonstration that transgenes that encode mutant neurofilament subunits can directly cause the selective degeneration and death of motor neurons, and the ensuing axonal disorganization (FIG. 4c,d), led to the proposal that damage to neurofilaments was directly involved in the pathogenesis of ALS[65,66]. A series of genetic efforts in mice has now shown that neurofilament content and organization strongly influence disease induced by mutant SOD1[61,62,66–69]. Eliminating neurofilaments by deletion of the major subunit NF-L (neurofilament light polypeptide) extends the lifespan of SOD1 mutant mice by 5–6 weeks, despite a loss early in postnatal development of 15% of the normal number of motor neurons in these mice[61]. Elevating the synthesis of the NF-L[69] or NF-H (neurofilament heavy polypeptide) subunits[62] powerfully slows SOD1-mutant-mediated disease (TABLE 2). All of these genetic manipulations lead to elevation of the number of neurofilament subunits in motor neuron cell bodies, and the most effective ones sharply reduce axonal neurofilament content. These observations have led to two competing explanations. On the one hand[62,68,69], increased NF-M (neurofilament medium polypeptide) and NF-H contents in perikarya might provide a further buffer against a cascade of aberrant perikaryal events, including increased calcium levels that arise from errors in glutamate handling[62] or inappropriate activation[68] of the prominent neuronal cyclin-dependent kinase CDK5. On the other hand[61,70], the speed of slow axonal transport is known to be slowed by neurofilaments[71]. So, as one of the earliest cellular abnormalities in motor neurons of mice that express mutant SOD1 is a reduction in slow transport[70], reducing the axonal load of neurofilaments might counterbalance the toxicity of the SOD1 mutants. Viewing all of the evidence, it seems most likely that both explanations are important contributors.

Are direct mutations in neurofilaments causes of or contributors to human ALS? Examination[72–74] of the repetitive tail domain of the large neurofilament subunit NF-H, especially in the extensive study of Al-Chalabi *et al.*[72], has identified a set of small in-frame deletions or

PENETRANCE
The proportion of genotypically mutant organisms that show the mutant phenotype. If all genotypically mutant individuals show the mutant phenotype, then the genotype is said to be completely penetrant.

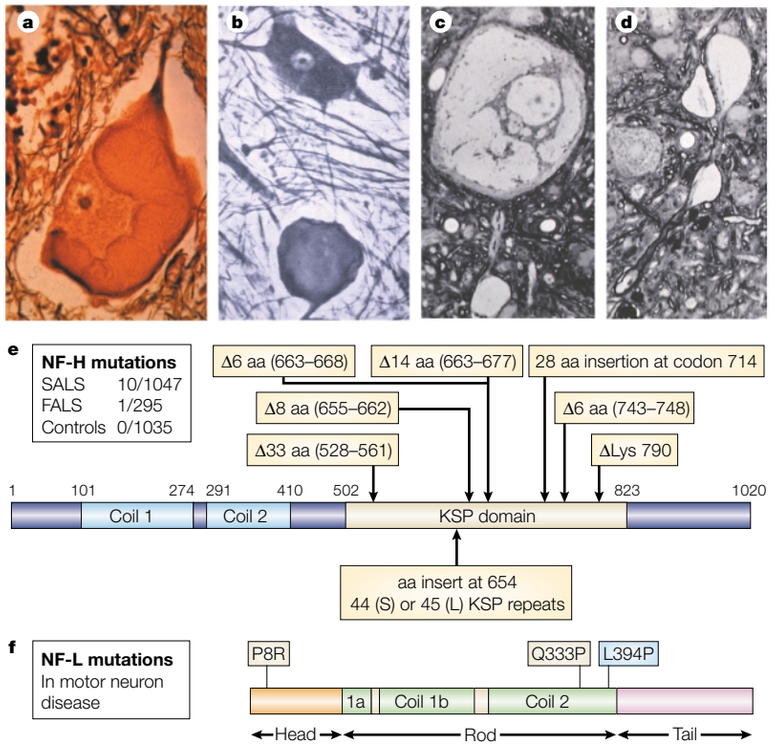


Figure 4 | **Neurofilament involvement in motor neuron disease. a**,**b** | Aberrant accumulation of neurofilaments is a common feature in motor neuron cell bodies and axonal swellings of sporadic amyotrophic lateral sclerosis (ALS). **a** | Silver staining reveals swirling masses of neurofilaments swelling a motor neuron cell body in the spinal cord of a sporadic ALS patient. **b** | A spheroidal swelling comprising masses of tangled neurofilaments in a proximal axonal segment of a spinal motor neuron from a sporadic ALS patient[64]. Note that the cell body of this neuron appears to be normal. **c**,**d** | Disorganized neurofilaments in the cell body (**c**) and spheroidal axonal swellings (**c**,**d**) of a mouse that has developed motor neuron disease by expressing a point mutation (NF-L$^{L394P}$) in the neurofilament light polypeptide (NF-L) gene (reproduced with permission from REF. 79 © 1994 Elsevier Science). **e** | Deletions or insertions identified in the KSP (lysine-serine-proline) repeat domain of the neurofilament heavy polypeptide (NF-H) gene from sporadic (SALS) and familial (FALS) ALS patients. Deletions or insertions of amino acids (aa) were identified as follows: at position 528 and 790 by Figlewicz *et al.*[73]; at 655, 663, and 743 by Al-Chalabi *et al.*[72]; and at 714 by Tomkins *et al.*[74]. **f** | Mutations in the NF-L gene that are known to be primary causes of motor neuron disease. Two mutations in human NF-L (P8R and Q333P) are causes[75,76] of some instances of a dominantly inherited motor neuropathy, Charcot–Marie–Tooth disease type II. Mutation NF-L$^{L394P}$ has been shown to cause dominant motor neuron disease in mice[79].

insertions in ~1% of more than 1,300 ALS patients; almost all of these mutations appear in 'sporadic' cases (FIG. 4e). Search in this one domain alone has therefore yielded mutations in the overall patient population that are about half as frequent as SOD1 mutations. Although the known neurofilament sequence variants are surely not capable by themselves of provoking disease with high PENETRANCE, the collective evidence now strongly indicates that variants in neurofilaments are at least important risk factors for apparently sporadic disease. This is further strengthened by the discovery (see FIG. 4f) that a dominant mutation in NF-L is a primary cause of one form of the motor neuropathy — CHARCOT–MARIE–TOOTH DISEASE[75,76]. Together with the role of neurofilaments in determining axonal diameter[77], and the realization that only the largest-calibre, neurofilament-rich axons are lost in human ALS[78] or SOD1-mutant-mediated disease in mice[79], the simplest view is that mutant neurofilament content and/or disorganized neurofilaments represent risk factors that underlie selective vulnerability.

CHARCOT–MARIE–TOOTH DISEASE
A genetic neurological disease that is characterized by the degeneration of peripheral nerves and a slowly progressive atrophy of the muscles in the foot, lower leg, hand and forearm, and a mild loss of sensation in the limbs, fingers and toes. The first sign of the disease is generally a high-arched foot and gait disturbances.

### Excitotoxicity in ALS

Glutamate-mediated excitotoxicity from repetitive firing and/or elevation of intracellular calcium by calcium-permeable glutamate receptors has long been implicated in neuronal death. Glutamate released from presynaptic terminals triggers action potentials in motor neurons by diffusing across the synaptic cleft to activate specific receptors on the dendrites of the postsynaptic neuron. A key component of the mechanisms necessary to prevent repetitive firing is the rapid removal of synaptic glutamate by glutamate transporters. Five subtypes of transporter are known, but it is the glial glutamate transporter EAAT2 that is responsible for ~90% of the clearance for motor neurons[59,80]. Evidence of abnormalities in glutamate handling in ALS arose from the discovery of large increases in the levels of glutamate in the cerebrospinal fluid of ALS patients[81–83], a finding now reported in ~40% of sporadic ALS patients[84]. Direct measurement of functional glutamate transport in ALS revealed a marked diminution in the affected brain regions, which was the result of a pronounced loss of the astroglial EAAT2 protein[85]. Although evidence for EAAT2 loss through RNA missplicing in astrocytes[86] has not been confirmed in subsequent studies[87,88], lowering EAAT2 levels with an antisense oligonucleotide has shown that loss of transport activity directly induces neuronal death[59].

Glutamate excitotoxicity has been implicated in at least two other neurological diseases that bear resemblance to ALS[89,90]. In both of them, one in Guam and the other on the Kii peninsula of Japan, disease has been linked to a chronic reliance on native foods that are rich in glutamate receptor agonists. Together with the finding that the expression of at least two SOD1 mutants in mice leads to the functional loss of EAAT2 (REF. 91), it seems highly likely that astrocytic dysfunction, including deficits in EAAT2, mediates at least some of the toxicity to motor neurons in both inherited and sporadic ALS.

### VEGF: another risk factor in ALS?

A completely unexpected contributor to motor neuron survival emerged from the discovery that ALS-like symptoms and neuropathology can be produced in mice bearing a targeted deletion that eliminates the ability of the vascular endothelial cell growth factor (VEGF) gene to respond to tissue hypoxia[92]. VEGF has long been recognized as a crucial factor in controlling the growth and permeability of blood vessels. Hypoxia-induced expression of VEGF through transcription factors that respond to low oxygen tension is crucial for maintaining or restoring the vascular perfusion of normal tissues, and for triggering the ingrowth of blood vessels to supply the extraordinary metabolic demands of tumours. Targeted deletion of the hypoxia-response element in the VEGF gene resulted in mice with a normal baseline expression of VEGF, but with a pronounced deficit in the ability to induce VEGF in response to

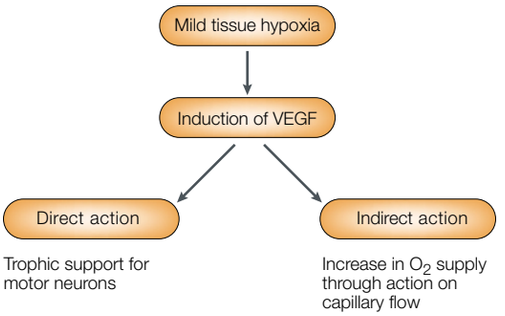


Figure 5 | **Models for the action of VEGF in supporting motor neuron survival.** Vascular endothelial cell growth factor (VEGF) and its induction by mild hypoxia might provide direct trophic support for motor neurons. It might also increase the oxygen supply available to motor neurons through its ability to increase capillary flow.

hypoxia. The unexpected finding was that the subset of mice that survived early development without hypoxia-dependent VEGF expression developed profound motor deficits that first appeared between 5 and 7 months of age and gradually progressed. All the classic features of ALS were observed: accumulation of neurofilaments in spinal cord and brainstem motor neurons, degeneration of motor axons and the characteristic denervation-induced muscle atrophy.

A requirement for inducible expression of VEGF in motor neuron health could arise from two novel mechanisms, acting alone or in combination (FIG. 5). The first is direct: VEGF might act directly on motor neurons as a neurotrophic or neuroprotective factor. Evidence for this includes the fact that VEGF can support the survival of primary motor neurons or a motor-neuron-like cell line *in vitro*, and can protect these cells from cell death induced by hypoxia or serum deprivation[92]. The second possibility emphasizes the traditional role of VEGF as a regulator of blood supply. The fact that motor neurons might be unusually sensitive to mild insufficiency in blood supply is not only experimentally verified in mice that cannot induce VEGF in response to hypoxia, but is easily understood considering the extraordinary metabolic burden placed on these cells, which consume high levels of energy to maintain a high rate of electrical firing. Motor neurons are also very large cells, and must transport almost all of their proteins and other macromolecules for long distances along their axons (up to a metre in length in humans). It seems most likely that the dual roles of VEGF in motor neuron biology explain, at least in part, the selectivity of disease in ALS. Moreover, this has profound implications in terms of environmental and genetic influences that could initiate or promote instances of sporadic ALS.

## Juvenile ALS from loss of a putative GEF

Since the discovery[3] of mutations in SOD1 as a primary cause of ~20% of dominantly inherited ALS, it has been disappointing that similar gene-mapping methods have not revealed the identities of genes affected in other familial examples of the disease. Why these genes have proved to be so elusive remains unknown, but the simplest view is that there are several different genes in which mutations can give rise to a similar disease, confounding chromosome-mapping efforts. However, loci for three milder motor neuron disorders have been identified (TABLE 1). These include one example of a syndrome with dominant inheritance and apparently complete penetrance, very juvenile onset (in the late teens), and a very slow progression of distal limb atrophy and motor neuron loss[93] that does not reduce lifespan. The locus has been mapped to chromosome 9q34, but the affected gene has not yet been identified[94].

Two other ALS-like disorders with recessive inheritance have been identified. The more common one is characterized by atrophy and weakness that initially affect hands and feet, with later onset of upper motor neuron involvement. This has been found in seven Tunisian families and the gene locus lies at 15q15–22, but the affected gene has not been identified with precision[95]. However, genetic studies have provided a powerful new insight into the mechanism of selective motor neuron death in a disease that is represented by a tiny pool of known instances arising in a handful of families. Originally mapped to chromosome 2q33, the affected gene (called *ALS2*) has been found to encode a 184-kDa protein derived from 34 exons that span 80 kb (REFS 96,97). The evidence that this gene is the ALS2 gene is compelling: single- or double-base deletions generate homozygous, frameshift mutants in each of the affected individuals. In one family, the frameshift leaves only a 46-amino-acid fragment, without any of the identifiable functional motifs. So, the disease arises in this case from what is, to all intents and purposes, a null mutant, strongly supporting the view that ALS2 arises simply from the loss of function of the affected gene.

The surface has barely been scratched in determining how loss of the ALS2 product leads to selective deficits in motor neurons. Sequence inspection reveals that the 650 amino acids of the amino-terminal of ALS2 contain all of the sequence motifs that are characteristic of GEFs. Many GEFs are known in biology; each is needed to recycle a specific, small G protein from its GDP-bound state to its GTP state. There is no information so far as to which G protein is the partner for this putative GEF. Its sequence homology is highest to a well-studied GEF named RCC1 (regulator of chromosome condensation), which acts on RAN, a G protein required for nuclear import and export. The carboxy-terminal half of ALS2 contains two further domains[96] — a PLECKSTRIN HOMOLOGY DOMAIN and a DBL HOMOLOGY DOMAIN — which are reminiscent of the RHO G-protein family that modulates dynamic actin assembly. Like SOD1, ALS2 is probably widely expressed, as indicated by the ubiquitous presence of two RNAs derived from it, the shorter of which encodes the GEF domain without the downstream two-thirds of the full-length protein. In the one family in which the mutation leaves unchanged the polypeptide that is encoded by the shorter form, but eliminates the larger polypeptide, the disease is restricted to upper motor neurons without

PLECKSTRIN HOMOLOGY DOMAIN
A sequence of about 100 amino acids that is present in many signalling molecules. Pleckstrin is a protein of unknown function that was originally identified in platelets. It is a principal substrate of protein kinase C.

DBL HOMOLOGY DOMAIN
A sequence that is present in some guanine nucleotide exchange factors, originally identified in the protein DBL, a molecule of unknown function that was originally identified in a human diffuse B-cell lymphoma.

RHO
A Ras-related GTPase involved in controlling the polymerization of actin.

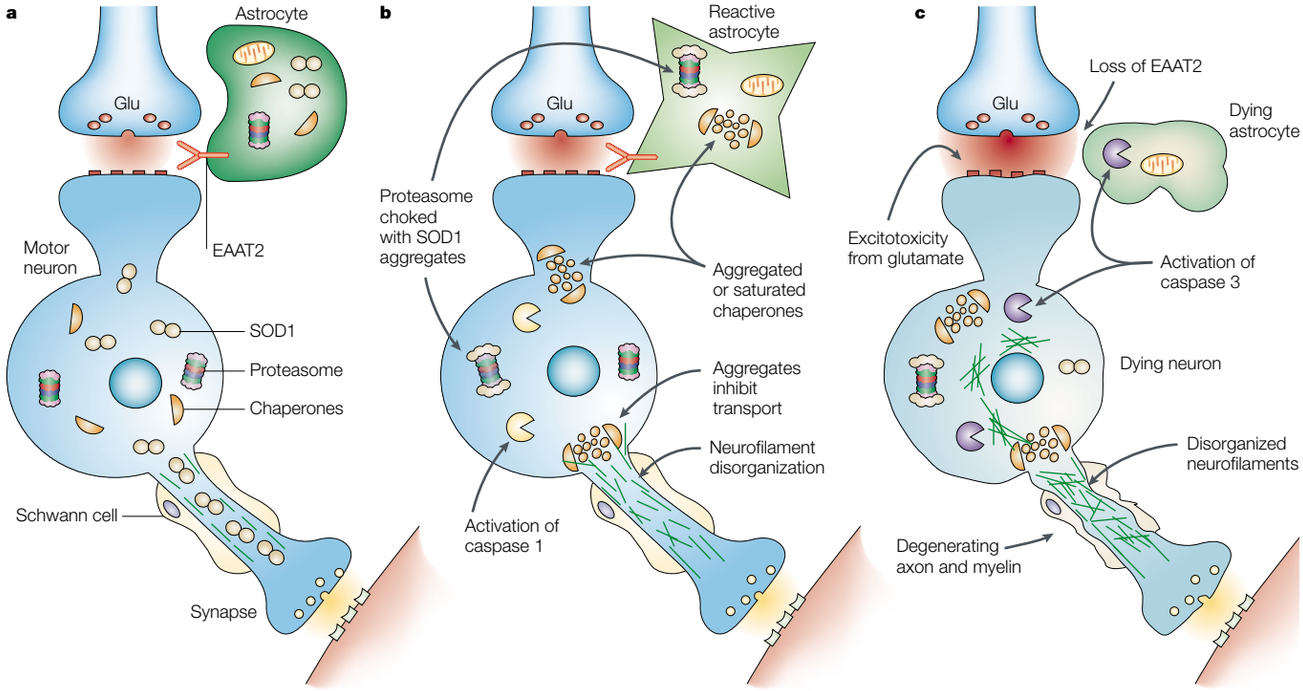


Figure 6 | **The specificity of the toxic effect of SOD1 mutations on motor neurons arises from the convergence of several risk factors. a** | Initially, a lower motor neuron receives signals to fire by the release of glutamate (Glu) from an upstream neuron, either an upper motor neuron or an interneuron. This signal is converted within the motor neuron into action potentials that stimulate the release of acetylcholine (orange) at its axon terminus, triggering muscle contraction. **b** | In neurons and astrocytes, superoxide dismutase 1 (SOD1) accumulates during aging, forming mutant SOD1 aggregates, either by an inherently unstable conformation or by self-induced oxidative damage. This triggers a loss of overall protein-folding chaperone activity and inhibits the removal of other damaged proteins by choking the 20S proteasome. Neurofilaments, especially in axons, become disorganized, inhibiting transport of components along the axon. Caspase 1 is chronically activated. **c** | Inhibition of chaperone and proteasome activity, loss of axonal transport capacity and an accelerated SOD1-mutant burden force chronic deficits in motor neurons. Similar damage in astrocytes suppresses the accumulation and activity of glutamate transporters (EAAT2) that are necessary for recovering synaptic glutamate and for preventing repetitive motor neuron firing. Such disproportionate firing produces excessive calcium entry through calcium-permeable glutamate receptors, activating caspase 3, which serves as the executioner for motor neuron death through the degradation of key cellular components.

*Animated online*

affecting lower motor neurons[97]. This produces a milder disease known as primary lateral sclerosis (or PLS). An intense search is now underway in seeking to identify what G-protein-mediated events are affected by the loss of one or both ALS2 polypeptides.

### Modelling toxicity in ALS

In considering what lessons have now been learned about the mechanisms underlying ALS, the evidence at first glance seems to support a discouraging series of divergent possibilities. However, it seems to us that, rather than viewing the potential contributors as alternatives, the timing and selectivity of motor neuron killing probably arises from the unfortunate convergence of a series of factors, all of which are necessary to place motor neurons at risk (FIG. 6). For familial ALS, a toxic property of mutant SOD1 probably arises from mutant-dependent, aberrant, copper-mediated chemistry and/or protein misfolding that disrupts chaperone activity and/or proteolysis by the proteasome (FIG. 6b). Intracytoplasmic aggregates of mutant SOD1 are common features of the disease, and might reflect primary aspects of toxicity, either through strangulation of axonal transport or by direct damage to astrocytes, perhaps inducing errors in messenger RNA maturation and glutamate metabolism. Such protein misfolding/aggregation represents a property common to the ALS-linked mutants, and is manifest at very early times in both neurons and astrocytes. The degree to which disease is caused by toxicity in either cell type has yet to be established, but the timing of onset or speed of disease progression probably arise from damage accumulated within both cell types.

Deficits in slow axonal transport arise early on[70], both as a secondary consequence of damage to other cell components and through direct targeting of SOD1 toxicity to molecular motors or cargoes, especially neurofilaments. Neurofilament mutations can be direct causes of motor neuron disease in mice[79], and their misorganization by mutation or other covalent damage might also force chronic deficits in axonal transport, which provoke the initiation of a further cascade of death events. Damage within astrocytes inhibits the accumulation[19] and activity of the main glutamate transporter EAAT2, allowing excessive firing of motor neurons (FIG. 6b). Being naturally depleted in cytoplasmic calcium-binding proteins such as calbindin[98], higher rates of neuronal firing become selectively excitotoxic for motor neurons that express calcium-permeable glutamate receptors. Damage is accelerated by the

natural increase in SOD1 in the spinal cord during aging[19], triggering the activation of a cascade of caspases, beginning with caspase 1, months before the onset of neuronal death[27]. Ultimately, the convergence of toxic properties and risk factors, none of which alone is sufficient to provoke neuronal death, triggers the activation of the executioner caspase 3 as the last step in the toxic cascade[26,27].

What about the 98% of disease cases that do not arise from SOD1 mutations? The convergence model, including a common set of risk factors, might well prove to be correct here too. The key difference would be the substitution of mutant SOD1 with a further combination of initiating genetic or environmental modifiers. Other possibilities to be anticipated include the identification of additional genes, individual variants of which can modify toxicity. Such modifiers are sure to exist. The mutant SOD1$^{D90A}$ is frequent in Scandinavia, but it induces disease in this population only when homozygous[12]. In other ethnic backgrounds, this mutant is like all the rest, causing disease in a dominant manner. The modifier locus is tightly linked to the *SOD1* gene itself[99]. A simple view is that variation in some non-coding portion of the *SOD1* gene affects its expression levels in motor neurons during aging. Other genetic contributors are just beginning to be identified: an important step has been the identification of one dominant locus on mouse chromosome 13, which can sharply slow the initiation of SOD1-mutant-mediated disease[100].

## Therapies: a cocktail of drugs and stem cells

Over the past two decades, there have been a large number of double-blind, placebo-controlled trials in ALS, involving several-thousand ALS patients (TABLE 2). Most have been based on efficacy in earlier animal models (including the WOBBLER MOUSE, the NEUROMUSCULAR DEGENERATION MOUSE and the PROGRESSIVE MOTOR NEUROPATHY MOUSE), but each has failed in the human trial. This includes trials with brain-derived neurotrophic factor (BDNF)[101], ciliary neurotrophic factor (CNTF)[102] and insulin-like growth factor 1 (IGF1)[103,104]. An initial trial of glial-derived neurotrophic factor (GDNF) was halted during phase I safety studies. All of these trophic factors were subsequently found to be ineffective (less than a 15% effect on survival) in the SOD1$^{G93A}$ mouse. From these parallel studies in mice and men, there is a growing consensus that the earlier mouse models have little in common with ALS and should not be used as models of the disease. Rather, agents that provide life extension to the SOD1 mutant mice should be studied in ALS patients, whereas, conversely, an agent that fails in the SOD1 mice should probably not be studied in large clinical trials.

So far, only one drug, riluzole, which is thought to act in part by limiting glutamate release, has been approved in the United States for use in ALS. This decision came in 1995, after two independent clinical trials in which riluzole was found to marginally increase the survival of ALS patients[105,106]. Unfortunately, patients taking this drug experience no obvious slowing in their loss of strength over time, and no improvement in muscle function. Despite its limited efficacy, the results with riluzole support an important component of glutamate toxicity in the pathway of cell death in ALS.

Future interventions can now be imagined at several levels. For example, agents that stimulate glutamate transport activity, either by influencing the levels of EAAT2 or by increasing the fraction of time that transporters are actively transporting. Alternatives would include a search for agents that affect neurofilament synthesis or assembly to simulate the remarkable delay in disease onset seen in several SOD1 mutant mice. Other potential directions involve agents that interfere with caspase-mediated cell death; this approach is supported by an extended lifespan in SOD1 mutant mice after chronic elevation of the apoptosis inhibitor Bcl2 (REF. 24) or intraventricular administration of a broad-spectrum caspase inhibitor[26]. And we should not forget the extended lifespan seen in SOD1 mutant mice by the addition of creatine to drinking water[107]. The mechanism of neuroprotection is not known, but might include mitochondrial energy metabolism, glutamate transport and/or direct effects on atrophic muscle[107,108]. Out of more than 40 different drugs and molecular therapies reported in SOD1 mice (TABLE 2), the addition of creatine remains the easiest 'pharmacological' intervention. How creatine provides this benefit remains unclear, but the outcome of at least two controlled human trials for creatine is anticipated next year.

Beyond these interventions, the long-term prospect with the highest visibility is the stem cell replacement of lost or degenerating neurons. No such attempts have yet been reported in ALS. However, the challenges for stem cell therapy in a diffuse disease like ALS are daunting; they include the appropriate differentiation of stem cells into motor neurons (either *in vitro* before grafting or locally in the spinal cord after implantation), prevention of the normal apoptosis that fetal motor neurons undergo when not in contact with muscles, establishment of appropriate connections from descending upper motor neurons and intereurons, appropriate regrowth/targeting of motor axons out of the spinal cord to the distant muscle, immunorejection, and diffuse delivery of motor neurons throughout the spinal cord and motor cortex. But even the simple replacement of motor neurons (if it really were so simple!) would clearly be short-sighted, given the abundance of data indicating astroglial dysfunction in sporadic and familial ALS. So, functional replacement might be more helpful by restoring healthy astrocytes with normal (or enhanced) glutamate transporter capacity. Even more simple in concept, the initial uses of such approaches might be most effective if focused on the use of stem cells as delivery tools for trophic support to existing motor neurons, and as a neuroprotectant for inactivating excess glutamate. But it should be obvious that real recovery of strength and potential for reversal of the disease could come only from replacement therapy — the replacement of dead/dying motor neurons and dysfunctional astrocytes. Stem cell therapy therefore offers the greatest hope — and the greatest challenge.

WOBBLER MOUSE
A mutant mouse that shows motor neuron degeneration and astrocyte reactivity in the spinal cord, and defects in spermatogenesis. The mutant gene has not been identified.

NEUROMUSCULAR DEGENERATION MOUSE
A mutant mouse that shows severe muscle atrophy due to progressive degeneration of spinal motor neurons. The responsible mutation has been localized to a gene known as *Smbp2*, which encodes a DNA-binding protein.

PROGRESSIVE MOTOR NEUROPATHY MOUSE
A mouse with an autosomal-recessive mutation that results in early-onset motor neuron disease, rapidly progressing hindlimb paralysis, severe muscular wasting and death. The mutant gene has not been identified.

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## Online links

**DATABASES**

**The following terms in this article are linked online to:**

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