# Molecular biology of amyotrophic lateral sclerosis: insights from genetics

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Abstract | Amyotrophic lateral sclerosis (ALS) is a paralytic disorder caused by motor neuron degeneration. Mutations in more than 50 human genes cause diverse types of motor neuron pathology. Moreover, defects in five Mendelian genes lead to motor neuron disease, with two mutations reproducing the ALS phenotype. Analyses of these genetic effects have generated new insights into the diverse molecular pathways involved in ALS pathogenesis. Here, we present an overview of the mechanisms for motor neuron death and of the role of non-neuronal cells in ALS.

Orphan disease
A condition that affects fewer than 200,000 people nationwide.

Fasciculation
A muscle contraction visible under the skin that represents the spontaneous firing of a single motor neuron and, as a result, of all the muscle fibres it innervates.

Spasticity
Persistent muscle contraction that causes stiffness and interferes with gait, movements and speech.

Bunina bodies
Characteristic proteinacious inclusions in ALS motor neurons.

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Amyotrophic lateral sclerosis (ALS; also known as Lou Gehrig's disease and motor neuron disease) is a progressive, lethal, degenerative disorder of motor neurons. The hallmark of this disease is the selective death of motor neurons in the brain and spinal cord, leading to paralysis of voluntary muscles[1]. The paralysis begins focally and disseminates in a pattern that suggests that degeneration is spreading among contiguous pools of motor neurons. Currently, there are approximately 25,000 patients with ALS in the USA, with a median age of onset of 55 years. The incidence and prevalence of ALS are 1–2 and 4–6 per 100,000 each year, respectively, with a lifetime ALS risk of 1/600 to 1/1,000 (REFS 2,3). ALS is therefore an orphan disease, although its uniform lethality imparts an importance out of proportion to its prevalence. Although most cases are classed as sporadic ALS (SALS)[4], 10% of cases are inherited (known as familial ALS; FALS). Age and gender are documented SALS risk factors[5] (the male: female ratio is 3:2).

In both SALS and FALS, there are progressive manifestations of dysfunction of lower motor neurons (atrophy, cramps and fasciculations) and cortical motor neurons (spasticity and pathological reflexes) in the absence of sensory symptoms[1,3]. However, muscles that control eye movements and the urinary sphincters are spared[3]. Respiratory failure causes death, which typically occurs within five years of developing this debilitating condition. The pathological hallmark of ALS is the atrophy of dying motor neurons. Swelling of the perikarya and proximal axons is also observed, as is the accumulation of phosphorylated neurofilaments, Bunina bodies and Lewy body-like inclusions, and the deposition of inclusions (spheroids) and strands of ubiquitinated material[6] in these axons. In addition, the activation and proliferation of astrocytes and microglia[6] are also common in ALS. Regrettably, there is no primary therapy for this disorder, and the single drug approved for use in ALS, riluzole, only slightly prolongs survival. Symptomatic measures (for example, feeding tube and respiratory support) are the mainstay of management of this disorder.

The causes of most cases of ALS are as yet undefined. Investigations have identified multiple perturbations of cellular function in ALS motor neurons, incriminating excessive excitatory tone, protein misfolding, impaired energy production, abnormal calcium metabolism, altered axonal transport and activation of proteases and nucleases[7,8]. Several factors are proposed to instigate these phenomena, including latent infections by viral and non-viral agents[9,10], toxins (for example, insecticides and pesticides) and autoimmune reactions[8]. Recent studies of inherited ALS have extended the understanding of the pathophysiology of this disease and approaches to its treatment. Here, we discuss the broad physiological implications of Mendelian, mitochondrial and complex genetic defects in ALS, and present an overview of how new knowledge of this disease can generate new strategies in ALS therapy.

## Mendelian genetics of ALS

Five Mendelian gene defects have been reported to cause ALS (TABLE 1). The protein products of these mutated genes are cytosolic Cu/Zn superoxide dismutase (SOD1)[11], alsin[12,13], senataxin (SETX)[14,15], synaptobrevin/VAMP (vesicle-associated membrane protein)-associated protein B (VAPB)[16] and dynactin[17]. Additionally, loci have been identified for ALS (on chromosomes 15, 16, 18, 20 and X)[18–23] and for ALS with frontotemporal dementia (ALS-FTD)[24,25].

Table 1 | **Genes that predispose to ALS**

| ALS disease type | Gene | Chromosome | Inheritance | Clinical features |
|---|---|---|---|---|
| ***Mendelian genes*** | | | | |
| ALS1 | *SOD1* | 21q22 | AD | Typical ALS |
| ALS2 | *ALS2* | 2q33 | AR | Juvenile onset, slowly progressive, predominantly corticospinal |
| ALS4 | *SETX* | 9q34 | AD | Adult onset, slowly progressive |
| ALS8 | *VAPB* | 20q13 | AD | Typical ALS |
| ALS | Dynactin | 2p13 | AD | Adult onset, slowly progressive, early vocal cord paralysis |
| ***Mendelian loci*** | | | | |
| ALS5 | ? | 15q15–21 | AR | Juvenile onset, slowly progressive |
| ALS6 | ? | 16q12 | AD | Typical ALS |
| ALS7 | ? | 20p13 | AD | Typical ALS |
| ALS-FTD | ? | 9q21–22 | AD | ALS, frontotemporal dementia |
| ALS-FTD | ? | 9p | AD | ALS, frontotemporal dementia |
| ALS-X | ? | Xcen | XD | Typical ALS |
| ***Mitochondrial genes*** | | | | |
| ALS-M | *COX1* | mtDNA | Maternal | Single case, predominantly corticospinal |
| ALS-M | *IARS2* | mtDNA | Maternal | Single case, predominantly lower motor neuron |

Variants of the genes encoding for angiogenin, neurofilament light subunit, survival motor neuron and vascular endothelial growth factor have been implicated in sporadic ALS. AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AR, autosomal recessive; *ALS2*, the gene that encodes alsin; *COX1*, cyclooxygenase 1; FTD, frontotemporal dementia; *IARS2*, mitochondrial isoleucine tRNA synthetase; mtDNA, mitochondrial DNA; *SETX*, senataxin; *SOD1*, superoxide dismutase 1; *VAPB*, vesicle-associated membrane protein-associated protein B; Xcen, centromere on the X chromosome; XD, X-linked dominant.

***Superoxide dismutase 1.*** SOD1 is a ubiquitous, predominantly cytosolic protein consisting of 153 amino-acids that functions as a homodimer. Each subunit of SOD1 binds one zinc and one copper atom. Through cyclical reduction and oxidation (dismutation) of copper, SOD1 converts the superoxide anion, a by-product of oxidative phosphorylation in the mitochondrion, to hydrogen peroxide. About 20–25% of all FALS cases arise because of mutations in *SOD1*, the protein product of which accounts for 0.1–0.2% of the cellular proteins in the CNS. More than 125 mutations have been identified, spanning all five exons of *SOD1*; 114 cause disease, whereas six silent mutations and five intronic variants do not. Although most mutations are missense, 12 are nonsense or deletion mutations that produce a truncated protein[26] (see ALS Online Database in Online links box). These mutations are specific for FALS, and are infrequently found in SALS (~1% of cases). Although most mutations reduce dismutation activity, others retain full catalytic function. There is no clear correlation between enzyme activity, clinical progression and disease phenotype, although the duration of the disease is similar for any given mutation[27–29].

The missense mutations that lead to the A4V and D90A substitutions in the protein sequence produce distinctive phenotypes. The A4V mutation (the most common SOD1 mutation in North America) is associated with short survival (a mean of about one year) and limited upper motor neuron involvement[30]. In northern Scandinavia, 2–3% of the population is heterozygous for the D90A mutation, which is a benign polymorphism in that population[31]. However, individuals in that region who are homozygous for D90A develop slowly progressive motor neuron disease with prominent corticospinal signs and prolonged survival of more than a decade. By contrast, patients who are D90A heterozygotes outside the northern Sweden gene pool develop classic ALS with survival times of 3–5 years. It is proposed that the milder D90A/D90A phenotype in northern Scandinavia reflects the presence either of alleles that are tightly linked to the D90A variant that blunt the toxicity of this mutation, or of neuroprotective alleles throughout the genome in that population. The latter proposal is supported by the observation that another dominantly inherited neurodegenerative disorder, amyloidotic polyneuropathy, which is caused by the mutation of transthyretin, is less aggressive in Sweden than in other European or Japanese populations[32].

***Alsin.*** The *ALS2* gene comprises 34 exons that encode alsin, a 184 kDa protein[13]. Alsin contains three putative guanine nucleotide exchange factor (GEF) domains, involving Ras, Rab and Ran motifs. GTPases of the Ras subfamily regulate cellular signalling that couples extracellular signals to intracellular responses regulating vesicle transport and microtubule assembly. Alsin is ubiquitously expressed and is abundant in neurons, where it localizes to the cytosolic portion of the endosomal membrane[33].

The function of alsin is not fully understood, but it is known that it acts as an exchange factor for Rab5a *in vitro*, which regulates endosomal trafficking and Rac1 activity[34,35]. Interestingly, alsin suppresses mutant SOD1-mediated toxicity in immortalized motor neuron cell lines (NSC34) by binding to SOD1 through the RhoGEF domain[35]. Multiple different mutations have been identified in *ALS2*, including a recently found homozygous missense mutation[36]. Most mutations are

Ubiquitin
A ubiquitous protein present in all eukaryotes (but absent from prokaryotes). As part of the ubiquitin–proteasome complex, ubiquitin binds and labels proteins to be proteolytically digested and removed from the cell. The ubiquitin–proteasome system is essential for many cellular processes, including cell cycling, signal transduction and the regulation of gene expression.

Guanine nucleotide exchange factor
(GEF). A protein that mediates the exchange of GDP to GTP, catalysed by a GTP-binding protein.

GTPases
A large family of enzymes that bind and hydrolyse GTP.

predicted to truncate the protein[12,13], with the extent of alsin truncation varying with phenotype — alsin is less truncated in patients with milder phenotypes[12,13]. The findings that all alsin mutants are unstable[37] and that most patients are homozygous for the mutations indicate that this form of ALS is caused by a loss of function of alsin. Loss of alsin in mice does not trigger motor neuron degeneration and disease, but does predispose to oxidative stress[38], and causes age-dependent neurological defects and altered vesicle and endosome trafficking[39].

***Senataxin.*** Defects in SETX cause an autosomal dominant, juvenile onset motor neuron disease with distal muscle weakness and atrophy, normal sensation, pyramidal signs and a normal life-span. These individuals have missense mutations in *SETX*, which encodes a 303 kDa DNA/RNA helicase domain with homology to human *RENT1* and *IGHMBP2* — two genes that encode proteins involved in RNA processing[15,40]. Altered RNA processing is implicated in two other inherited motor neuron diseases — spinal muscular atrophy (with mutations in the survival motor neuron gene) or a severe, infantile, distal spinal muscular atrophy with prominent respiratory dysfunction (SMARD; spinal muscular atrophy with respiratory distress, with mutations in IGHMBP2).

***VAMP-associated protein B.*** Defects in this gene cause adult-onset, autosomal dominant ALS and atypical ALS (slowly progressive with tremors) but not frontotemporal dementia (for an example, see REF. 41). *VAPB* has six exons that encode a ubiquitously expressed 27.2 kDa homodimer, which belongs to a family of intracellular vesicle-associated/membrane-bound proteins that are presumed to regulate vesicle transport.

***Dynactin.*** Recently, dominantly transmitted mutations causing adult-onset, slow progressive, atypical motor neuron disorder have been identified in the p150 subunit of dynactin, a component of the dynein complex that comprises the major axonal retrograde motor[17]. The mutations in the p150 subunit appear to affect the binding of the dynactin–dynein motor to microtubules. The onset of this form of motor neuron degeneration is often heralded by vocal cord paralysis.

## Mitochondrial and complex genetics in ALS

***Mitochondrial gene defects.*** Defects in two mitochondrial genes cause motor neuron disorders with clinical features that are suggestive of ALS (TABLE 1). A 5 bp deletion in the mitochondrial gene cyclooxygenase 1 (*COX1*) results in early adult onset of corticospinal motor neuron loss[42]. By contrast, a 4272T>C mutation in mitochondrial transfer RNA (isoleucine) causes a late onset, slowly progressive, predominantly lower motor neuron disease[43].

***Complex genetics in ALS.*** There have been few studies of genetic variants that modify ALS susceptibility or phenotype. In DNA sets from Sweden, Belgium and Birmingham (UK)[44], but not London (UK)[44], Utrecht (The Netherlands)[45] or Boston (USA) (R.H.B., unpublished data), promoter polymorphisms that reduce the expression of vascular endothelial growth factor (VEGF) are associated with an increased risk of disease. Variants predicted to reduce the activity of another vascularizing factor, angiogenin (ANG), increase ALS risk selectively in Irish and Scottish DNA sets[46]. These observations support the view that vascularization and, indirectly, blood supply and cellular oxygen pressure are important determinants of motor neuron viability, or that VEGF and ANG exert direct neurotrophic influences on motor neurons. Polymorphisms in the genes that encode the neurofilament heavy subunit[47–49] and the survival motor neuron protein[50,51] are also incriminated as risk factors for development of ALS.

## Insights into ALS pathogenesis from genetics

Our understanding of the pathobiology of ALS is predicated largely on studies of ALS-associated gene mutations. Because the clinical and pathological profiles of sporadic and familial ALS are similar, it can be predicted that insights from studies of ALS-causing gene mutations apply to sporadic ALS. Most data on ALS pathogenesis are derived from studies of cell death initiated by mutant SOD1 protein. Mutations in *SOD1* and, as a result, its protein product initiate motor neuron disease through one or more toxic properties. This is consistent with observations that the inactivation of SOD1 does not cause fulminant motor neuron disease in mice[52], whereas transgenic mice that overexpress ALS-associated, mutant SOD1 proteins develop motor neuron disease despite having normal or elevated SOD1 activity[53]. Finally, neither age of onset nor rapidity of disease progression correlates with SOD1 activity in ALS patients[54]. Two broad hypotheses explain the adverse function of the mutant SOD1 protein (FIG. 1): either the mutant protein perturbs oxygen metabolism, or the primary problem is mutation-induced conformational instability and misfolding of the mutant peptide. In either case, perturbations of the biophysical properties of the SOD1 protein induce diverse pathogenic phenomena (FIG. 2).

***Aberrant chemistry and oxidative stress.*** The first hypothesis above proposes that mutations in SOD1 alter enzyme activities through either aberrant copper catalysis or improper metal binding. This is presumed to be a consequence of alterations in the configuration of the active channel that allow atypical substrates to interact promiscuously with copper. Mutant SOD1 might accept peroxynitrite[55] (formed by the spontaneous combination of superoxide and nitric oxide) or hydrogen peroxide[56] (the normal product of the first step of the dismutase reaction catalysed by SOD1) as a substrate, and thereby catalyse the nitration of tyrosine residues of SOD1 and hydroxyradicals[55,56].

The second hypothesis proposes that mutant SOD1 protein fails to bind zinc properly, allowing the rapid reduction of the SOD1-bound copper which, in its reduced state, catalyses the formation of superoxide anion rather than dismutation (so-called 'backward catalysis')[57]. Diminished metal binding by mutant SOD1

Endosome
A membrane-bound, intracellular oganelle. Endocytotic vesicles derived from the plasma membrane are actively transported to fuse with endosomes; endosomes also fuse with vesicles of the endoplasmic reticulum that contain newly expressed proteins.

Dynein
A motor protein that converts the chemical energy of ATP into mechanical energy for movement. Dynein transports several cellular cargos along the microtubules.

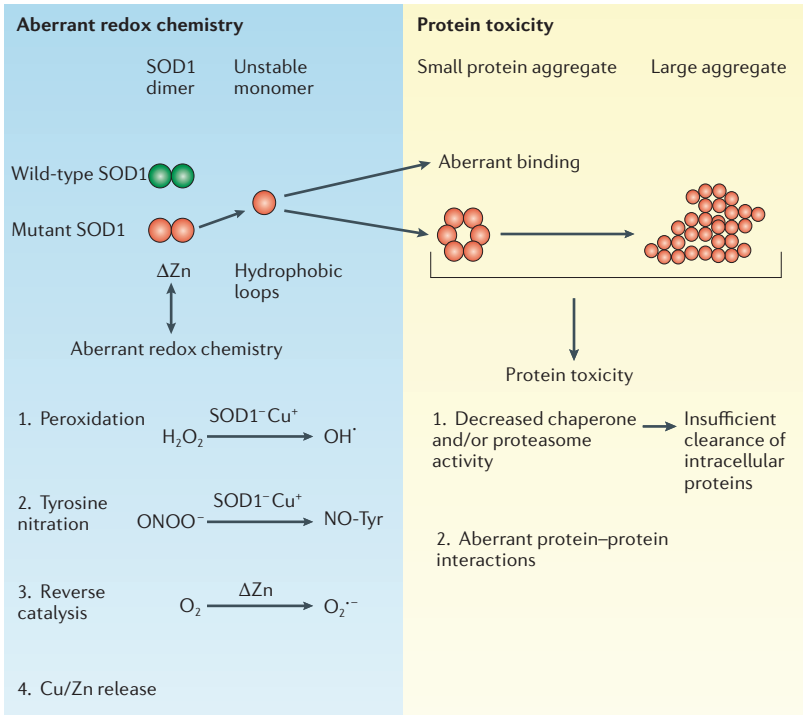


Figure 1 | **Models of mutant SOD1-mediated toxicity.** The instability of the mutant protein contributes to its toxicity, sometimes enhanced by the release of Zn. In the aberrant redox chemistry model, mutant superoxide dismutase 1 (SOD1) is unstable and the active channel opens, permitting aberrant chemistry through promiscuous interaction with non-conventional substrates. Hydrogen peroxide ($H_2O_2$) or nitronium ion ($ONOO^-$) can react with reduced SOD1 ($SOD1^-Cu^+$). Molecular oxygen ($O_2$) can react aberrantly with Zn-deficient SOD1 to generate an excess of superoxide anion ($O_2^{\cdot -}$). The unstable protein could also release free copper and/or zinc, which might be toxic. In the protein toxicity model, unstable, conformationally altered mutant SOD1 could form toxic, proteinaceous deposits. Aggregated SOD1 could inhibit chaperone and/or proteasome activity, with subsequent misfolding and insufficient clearance of numerous proteins. Alternatively, these aggregates could sequester, inactivate or enhance the toxicity of other proteins crucial for cellular processes.

could also enhance the release of copper and zinc, and trigger copper and/or zinc-mediated neurotoxicity.

The concept that mutant SOD1 provokes aberrant oxyradical reactions has been controversial. Some of the key findings have not been consistently detected; for example, increased levels of oxidative markers are evident in transgenic ALS G93A-SOD1 mice and patients with SALS[58,59] but not in transgenic G37R mice[60]. Another inconsistency is the impact of forced expression of wild-type *SOD1* jointly with mutant *SOD1* transgenes. In G85R mice, genetic manipulations of levels of wild-type SOD1 (elimination or forced expression at high levels) do not affect the age of onset, the survival or the rate of progression; these findings could be used to argue against a direct role of heightened oxygen-mediated toxicity in the degeneration of motor neurons[61]. By contrast, the forced expression of high levels of wild-type *SOD1* in G93A, L126Z and A4V-SOD1 mice accelerates the onset of the disease[62,63]. This effect has been attributed to the enhanced formation of mitochondrial aggregates containing both mutant and wild-type SOD1 (REF. 63).

Also of note is evidence that transgenic mice that lack a protein required to load copper into SOD1 possess only minute levels of SOD1-bound copper and have drastically reduced dismutation activity but still develop motor neuron disease[64]. Moreover, mice that harbour mutations in the histidines that bind copper in the SOD1 active site, which leads to loss of dismutase activity, also develop motor neuron disease[65]. Similarly, the G86R mutated murine form of SOD1 causes motor neuron disease in mice, even though a possible alternative copper-loading site, Cys111, is absent[66]. The most plausible interpretation is that copper-mediated oxyradical chemistry is not required for the motor neuron pathology provoked by mutant SOD1; a caveat is that the mutant SOD1 might exert aberrant catalysis through copper loaded in a copper chaperone for SOD1 (CCS)-independent fashion on the surface of the protein[67].

***Protein instability and SOD1 aggregation.*** Another set of hypotheses propose that the conformational instability of mutant SOD1 induces the formation of harmful aggregates. In transgenic rodents with SOD1-mediated ALS (including copper-binding-site-null mice) and in some human ALS cases, aggregates that are immunoreactive for SOD1 are detected in motor neurons, the neuropil and astrocytes[61,68]. In these transgenic mice, these aggregates become evident by the time of disease onset[69,70], and increase in abundance with disease progression[61,69,70].

*In vitro* studies have shown that, in contrast to stable, dimeric wild-type SOD1, the mutant proteins oligomerize over time to form small pore-like structures that are similar to some forms of β-amyloid protein[71,72]. Whether such protein structures *in vitro* correspond to structures in human ALS neural tissue *in vivo* is not known. In the transgenic animal models, the formation of non-native, sub-microscopic, detergent-insoluble SOD1 species is a common feature of all mutant SOD1 proteins. Because this is not the case for the large, intracellular aggregates, it could be that the less evident microscopic aggregates are the vital aggregated protein component in mutant SOD1-mediated ALS. Are SOD1-associated protein inclusions toxic? If so, why are they toxic? As in other neurodegenerative disorders, it remains unclear whether protein aggregates that are detected in the CNS are harmful. As in Huntington's disease, it might be that protein inclusions are favourable, perhaps because they sequester the mutant protein. By contrast, studies have reported a correlation between protein aggregation and a clinical phenotype of transgenic *SOD1* mice: mutations that cause a more severe disease (gauged by shortened survival) have a shorter half-life and the protein products are more likely to form aggregates[73,74]. There is considerable speculation about the potential toxicity of inclusions. It has been proposed that inclusions could both mediate oxyradical chemistry (see above) and overwhelm the proteasome. The latter is predicted to impair protein degradation and recycling (not only of mutant SOD1 but also of other proteins normally processed by proteasomes) and sequester proteins that are crucial for cellular processes, such as heat-shock proteins (HSPs). Mutant SOD1 directly interacts with HSPs, such as HSP70, HSP40, HSP27 and

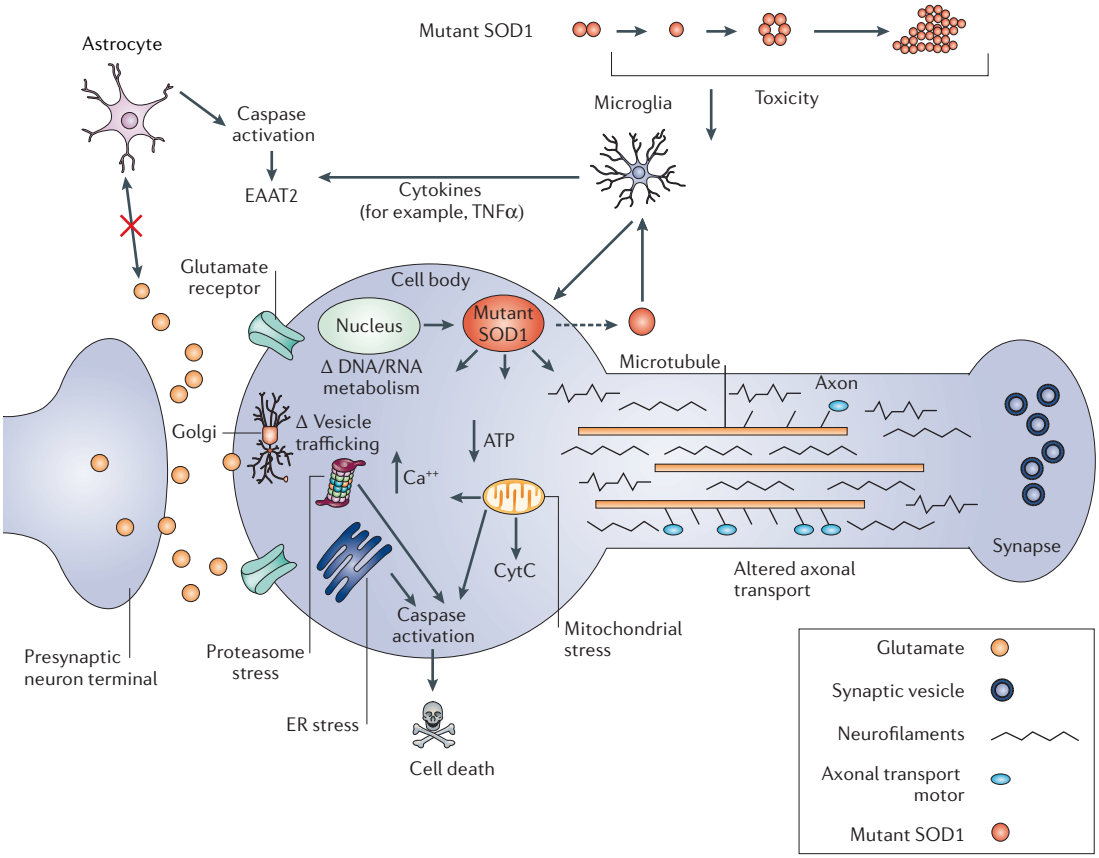


Figure 2 | **Mutant SOD1 impairs multiple cellular functions.** The toxicity of mutant superoxide dismutase 1 (SOD1) is multi-factorial, operating through diverse, interrelated pathways. Within the motor neuron, mutant SOD1 adversely affects DNA/RNA metabolism, mitochondria (diminishing ATP production and calcium buffering, increasing the release of free calcium), neurofilaments and axonal transport, and the function of the endoplasmic reticulum (ER), the Golgi apparatus and proteasomes. In turn, ER stress and apoptotic cascades are activated. Through an interaction with chromogranin (a secretory glycoprotein), mutant SOD1 could be secreted from the motor neuron. The pathological process also involves the activation and dysfunction of astrocytes and microglia. Astroglial uptake of glutamate is reduced, contributing to excessive extracellular glutamate and excitotoxicity. Microglia secrete cytokines such as tumour necrosis factor-α (TNFα) that could be toxic and cause cellular damage. So, the death process in motor neurons reflects a complex interplay between intrinsic (autonomous) and extrinsic (non-cell autonomous) phenomena. CytC, cytochrome *c*; EAAT2, excitatory amino acid transporter 2. Modified, with permission, from REF. 187 © (2006) Taylor and Francis.

BCL2
The founding member of a family of apoptosis-regulating proteins. Many BCL2 family members regulate mitochondria-dependent steps in cell death pathways, with some suppressing and others promoting the release of apoptogenic proteins from these organelles.

Apoptosis
A mode of cell death in which the cell triggers its own destruction by activating pre-programmed intracellular suicide machinery.

Caspases
A family of intracellular cysteine endopeptidases that have a key role in mammalian apoptosis. They cleave proteins at specific aspartate residues.

Astrogliosis
Proliferation and ramification of glial cells in response to brain damage.

Microgliosis
Proliferation and activation of microglial cells, which are the primary immune effector cells in the brain.

αβ-crystallin[75], perhaps impairing the chaperone and/or the anti-apoptotic function of these proteins. It is likely that mutant SOD1 also sequesters the anti-apoptotic protein BCL2 at the surface of mitochondria[76].

***Apoptosis in ALS.*** Biochemical markers of apoptosis can be detected in the terminal stages of human and transgenic ALS[77–85]. Early reports suggested that *SOD1* mutations transform SOD1 from an anti- to a pro-apoptotic protein. Cultured neuronal cells either transfected or microinjected with mutant SOD1 cDNAs die by apoptosis[78,79]. Mutant SOD1 protein is also pro-apoptotic in an ALS transgenic mouse model[79–83]. Additional evidence linking SOD1 to apoptosis comes from the impairment of the association of cytochrome *c* (an intermediate in the apoptosis pathway) with the inner membrane of the mitochondrion in ALS transgenic mice, and from the finding that a gradual reduction in intra-mitochondrial cytochrome *c* correlates with disease progression[84].

Activation of caspase 1 (CASP1) is the earliest molecular abnormality in the SOD1-G85R mice, occurring months before CASP3 activation, motor neuron death and clinical onset[79–89]. In the spinal cord, activated CASP3 is found in both motor neurons and astrocytes[80], where it cleaves the astroglial excitatory amino acid transporter 2 (EAAT2)[85]. In the G93A-SOD1 mice at least, the activation of another downstream caspase (CASP7) also coincides with disease onset[86]. In agreement with the hierarchical order of caspase activation, in the G93A-SOD1 mice, cytochrome *c* translocation to the cytosol and concomitant CASP9 activation follow CASP1 and precede CASP3 and CASP7 activation[86]. Because the activation of CASP1 has been involved in both apoptosis and inflammation, it is possible that prolonged activation is a consequence of (and exacerbates) chronic neural inflammation (astrogliosis and microgliosis) in these mice. Alternatively, in contrast to developmental apoptosis in which cell death proceeds rapidly, mutant SOD1-mediated early caspase activation might initiate a slowly

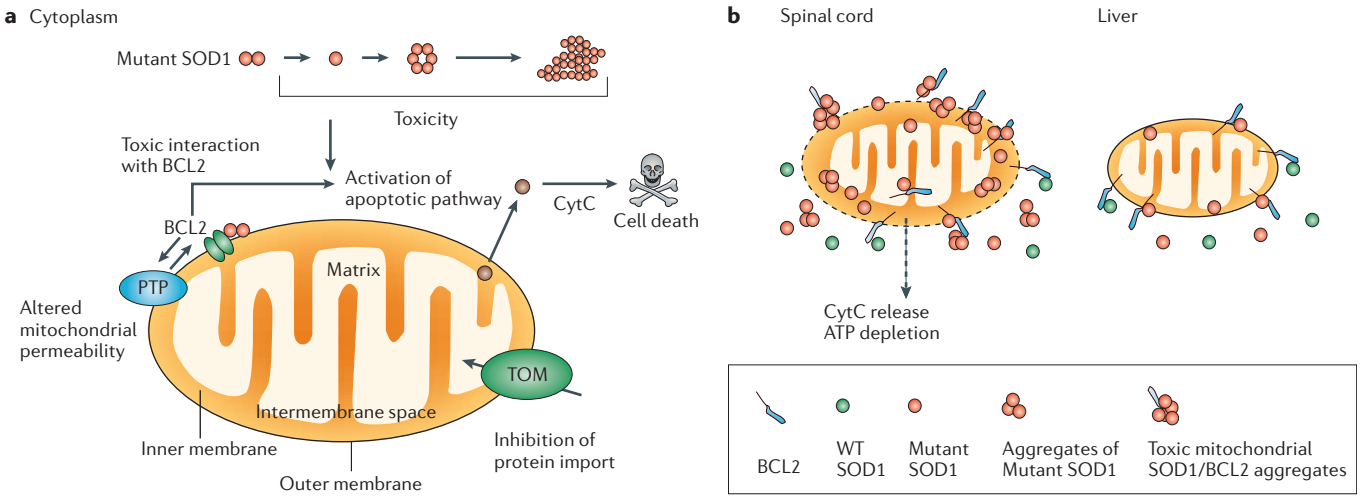


Figure 3 | **The mitochondrion as a target of mutant SOD1.** Long considered a cytoplasmic protein, superoxide dismutase 1 (SOD1) also localizes to the mitochondrion; its precise localization (outer membrane, intermembrane space or matrix) is not fully defined. **a** | Mutant SOD1 forms insoluble aggregates that could directly damage the mitochondrion through: swelling, with expansion and increased permeability of the outer membrane and intermembrane space, leading to release of cytochrome *c* (CytC) and caspase activation; inhibition of the translocator outer membrane (TOM) complex, preventing mitochondrial protein import; and aberrant interactions with mitochondrial proteins such as BCL2. **b** | Aggregates of mutant SOD1 and BCL2 are found specifically in spinal cord but not liver mitochondria, a finding that might relate to the motor neuronal specificity of mutant SOD1 phenotypes. Codeposition of mutant SOD1 with BCL2 might eliminate BCL2 function, disrupt the mitochondrial membrane, deplete energy, deregulate mitochondrial bioenergetics and activate the mitochondrial apoptotic pathway. WT, wild-type. Modified, with permission, from REF. 76 © (2004) Elsevier Science.

progressive cell death process that kills motor neurons and the surrounding cells over months and years.

Is this slow apoptotic process directly triggered by mutant SOD1? The gradual activation of an apoptotic pathway in ALS, and the anti-apoptotic influence of wild-type SOD1 (REF. 87), indicate that mutant SOD1 is directly involved in the initiation of caspase activation and cell death. The observations that both wild-type and mutant SOD1 bind the anti-apoptotic BCL2 and, more importantly, in so doing trap BCL2 into detergent resistant aggregates, are consistent with this view[76] (FIG. 3a,b). When entrapped in inclusions, BCL2 could be directly rendered non-functional. To the extent that BCL2 functions by binding other pro- and anti-apoptotic proteins, such segregation might also indirectly block BCL2 function. Yet another possibility is that, on binding to mutant SOD1, BCL2 undergoes conformational modification and becomes toxic. Entrapment and depletion of BCL2 is supported by studies that show reduced levels of BCL2 in SOD1 mice and patients with ALS. However, this is only pertinent to mutant SOD1 and does not explain the observed changes in BCL2 expression and function in non-SOD1 ALS cases.

Once the pro-apoptotic death signal has been generated, secondary events in the spinal cord amplify the disease process. These include the activation of microglia and T cells and the release of inflammatory factors and cytokines such as interleukin 1β, COX2 and tumour necrosis factor-α (TNFα)[88–91]. TNFα, which activates CASP8 in late stages of the disease in ALS transgenic mice[92,93], and its receptor are part of a superfamily of proteins that includes Fas. Cultured embryonic motor neurons are selectively sensitive to Fas-induced apoptosis, which suggests that these inflammatory/pro-apoptotic molecules mediate a motor neuron-specific apoptotic pathway and thereby selectively intoxicate motor neurons in ALS[92]. Another intriguing mechanism signalling neuroinflammation and toxicity is predicated on the accumulation of mutant but not wild-type SOD1 in the endoplasmic reticulum[94]. Results from recent studies show that mutant SOD1 interacts with proteins such as chromogranin in the endoplasmic reticulum and is thereby retained within that compartment, to be trafficked to the cell surface and secreted extracellularly[95]. According to this provocative model, once secreted, mutant SOD1 activates microglial and astrocytic cells to provoke a neuroinflammatory response around the motor neuron[95].

***Mitochondrial dysfunction in ALS.*** Numerous studies have focused on the role of the mitochondrion in the pathogenesis of neurodegenerative diseases like ALS. Evidence of mitochondrial dysfunction in ALS patients includes clusters of abnormal mitochondria and morphological defects identified within mitochondria in skeletal muscles and intramuscular nerves in human SALS cases[96,97]. In such cases biochemical analyses have delineated defects in the respiratory chain complexes I and IV in muscle[98] and elevated levels of mitochondrial calcium[99] in muscle and spinal cord. In ALS mice, the main morphological evidence for mitochondrial pathology is the presence of vacuolated mitochondria (for example, in the G93A and G37R transgenic lines). In the G93A-SOD1 mice, mitochondrial

Kinesins
A class of motor proteins that attach to microtubules and transport vesicles along the tubule.

vacuoles derived from a detachment between the inner and the outer membrane[100] are evident early, and drastically increase in both number and volume as the disease progresses[101–103].

*In vitro* and *in vivo* studies further define mitochondrial defects in ALS mice. The expression of mutant SOD1 in neuronal cell lines or in cultured primary motor neurons depolarizes mitochondria[104,105], impairs calcium homeostasis[106] and reduces ATP production[107]. Similarly, G93A-SOD1 mice show reduced respiratory chain activity and reduced ATP synthesis; these increase in severity as disease progresses.

Whether mitochondrial dysfunction and pathology represent primary or secondary pathological events is unknown, as mitochondrial abnormalities can both result from and cause oxidative toxicity. In either circumstance, the pathological mitochondria can mediate cell death by releasing calcium into the cytoplasm, producing inadequate levels of ATP and triggering apoptosis (FIG. 3b). It is likely that mitochondrial function modifies the course of motor neuron degeneration. In ALS mice, inhibition of manganese superoxide dismutase (a mitochondrial dismutase) accelerates disease progression. Conversely, the disease is slowed by interventions that improve mitochondrial function — for example, creatine, which inhibits opening of the mitochondrial transition pore, and minocycline, which blocks the egress of cytochrome *c*[108,109].

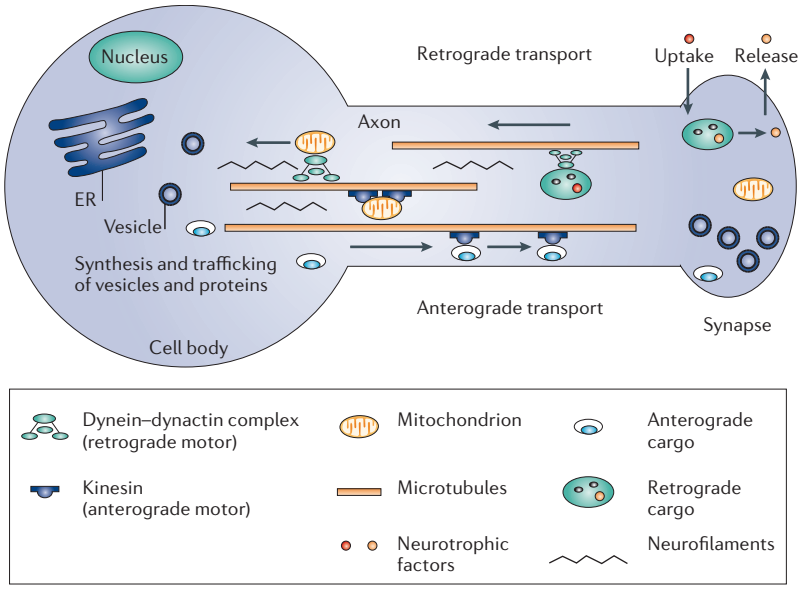


Figure 4 | **Axonal transport is abnormal in ALS.** The motors for anterograde and retrograde fast axonal transport are the kinesins and dynactin complex proteins, respectively; microtubules provide the tracks for these motors. Vesicles for transport are sorted and loaded onto transport motors both in the cell body and the distal nerve terminal. The former are transported not only into the axon but also into dendrites. Those in the distal nerve terminal permit uptake and axosomatic movement of substances such as trophic proteins. Mutations in dynactin (humans), dynein (mice) and three different forms of kinesin all provoke motor neuron degeneration. Perturbation of neurofilaments through mutations and changes in phosphorylation and the physical structure of the axon could also adversely affect axonal transport. In transgenic mouse models, mutant superoxide dismutase 1 (SOD1) impairs anterograde axonal transport. ER, endoplasmic reticulum.

During the past five years, several observations have indicated that mutant SOD1 could directly damage mitochondria. It is evident that a fraction of SOD1 is localized in the mitochondrion[76,110–113]. Studies of differentially targeted SOD1 *in vitro* reveal that mutant SOD1 more potently triggers cell death when localized to mitochondria and, once so targeted, forms intra-mitochondrial protein aggregates[114]. Several interlocking mechanisms explain how mutant SOD1 impairs mitochondria from within (FIG. 3a). In G93A-SOD1 mice, mutant SOD1 colocalizes with cytochrome *c* and the peroxisomal membranes associated with the vacuoles[100]. Therefore, mutant SOD1 could be involved in fusing the peroxisomes and the outer mitochondrial membrane, a process that could form pores in the mitochondrial membrane allowing the release of cytochrome *c* and the initiation of an apoptotic cascade. A second hypothesis is that mutant SOD1 is selectively recruited to the outer mitochondrial membrane, where it forms aggregates that slowly disrupt the protein translocation machinery (the translocator outer membrane (TOM) complex) of the mitochondrion and limits the import of functional proteins into the mitochondrion[112]. Two studies suggest that mutant SOD1 is selectively recruited to mitochondria only in affected tissues[76,112]. It has therefore been proposed that the selective loss of TOM function in spinal cord mitochondria might form the basis for the tissue specificity of ALS. Finally, mutant SOD1 aggregates could damage the mitochondrion through abnormal interaction with other mitochondrial proteins such as BCL2 (REF. 76). A recent paper challenged the notion of a toxic mitochondrial mutant SOD1, suggesting that mitochondrial loading of mutant SOD1 is simply the result of overexpression of the mutant SOD1 that normally does not associate with mitochondria[115].

***Altered axonal transport in ALS.*** Another characteristic of ALS is the reduced activity of axonal transport, described first in patients with ALS[116] and more recently in transgenic mouse models of ALS[117] (FIG. 4). The transport of molecules and organelles is a fundamental cellular process that is particularly important for the development, function and survival of neurons. This process is dictated by the highly polarized anatomy of neurons: axonal proteins are synthesized in the cell body and must be transported in an anterograde manner along the axons and dendrites to reach synapses, whereas substances such as peripherally located trophic factors must be transported centrally from the synaptic regions by retrograde transport. The molecular motors for anterograde and retrograde transport are kinesin and the dynein–dynactin complex, respectively. Transport is conventionally regarded as either slow or fast. Presumably, functional and efficient axonal transport is particularly important for motor neurons, which are among the largest and longest cells in the body.

Several findings indicate that defects in axonal transport might contribute to the demise of motor neurons in ALS. First, both slow and fast anterograde transport are slowed in transgenic G93A-SOD1 and G37R ALS mice prior to disease onset. These deficits are exacerbated

Table 2 | **Neuronal genes with defects that impair motor neuronal function or viability**

| Disease | Gene (gene symbol) | Inheritance | Class | Refs |
|---|---|---|---|---|
| ***ALS*** | | | | |
| ALS | Superoxide dismutase 1 (*SOD1*) | AD | Mainly cytosolic enzyme; also in mitochondrion | 11 |
| ALS | VAMP-associated protein B (*VAPB*) | AD | Golgi/vesicle transport and sorting factor | 16 |
| ***ALS-like*** | | | | |
| Slow ALS | Senataxin (*SETX*) | AD | DNA/RNA metabolism | 15 |
| jALS | Alsin (*ALS2*) | AR | GEF signalling | 12,13 |
| Slow ALS | Dynactin (*DCTN*) | AR | Transport motor | 17 |
| ***Peripheral neuropathy*** | | | | |
| CMT1F/2E | Neurofilament light chain (*NEFL*) | AD | Cytoskeletal filament | 154 |
| CMT2A1 | Kinesin K1F1Bβ (*K1F1Bβ*) | AD | Transport motor | 157 |
| CMT2A2 | Mitofusin (*MFN2*) | AD | Mitochondrial protein | 188 |
| CMT2B | RAB7 (*RAB7*) | AD | Golgi/vesicle transport and sorting factor | 160 |
| CMT2D | Glycyl-tRNA synthetase (*GARS*) | AD | DNA/RNA metabolism | 162 |
| CMT2F | Heat shock protein 27 (*HSP27*) | AD | Heat shock protein | 166 |
| CMT2L, scapuloperoneal neuropathy | Heat shock protein 22 (*HSP22*) | AD | Heat shock protein | 167 |
| DI-CMT 2B | Dynamin (*DNM2*) | AD | GTPase | 189 |
| DI-CMT, type C | Tyrosyl-tRNA synthetase (*YARS*) | AD | DNA/RNA metabolism | 163 |
| Hereditary sensory motor neuropathy | Rho GEF 10 (*ARHGF10*) | AD | GEF signalling | 190 |
| HSN1 | Serine palmitoyl transferase (*SPTLC1*) | AD | Enzyme | 191 |
| Porphyria | Porphobilinogen deaminase (*PBGD*) | AD | Enzyme | 192 |
| AR-CMT2A (and other phenotypes) | Lamin A/C (*LMNA*) | AD, AR | Nuclear membrane protein | 193,194 |
| Andermann syndrome | Potassium chloride co-transporter (*KCC3*) | AR | Cation-chloride cotransporter | 195 |
| Giant axonal neuropathy | Gigaxonin (*GAN*) | AR | Cytoskeletal filament | 155,156 |
| HSN4 | Tyrosine kinase A receptor/nerve growth factor receptor (*TRKAR/NGFR*) | AR | Growth factor receptor | 196 |
| Metachromatic leukodystrophy | Arylsulphatase A (*ASA*) | AR | Enzyme | 197 |
| Refsum's disease | Phytanoyl CoA hydroxylase (*PhyH*) | AR | Enzyme | 198 |
| Riley-Day (HSN3) | Inhibitor of κ light chain enhancer in B cells (*IKBKAP*) | AR | Transcription factor | 199 |
| Tangier sensory motor neuropathy | ABC1 (*ABC1*) | AR | ABC transporter | 200 |
| ***Lower motor neuropathy*** | | | | |
| X-linked spinobulbar muscular atrophy | Androgen receptor (*AR*) | AD | DNA/RNA metabolism | 201 |
| Congenital fibrosis of extraocular muscles | Kinesin 21A (*KIF21A*) | AD | Transport motor | 202 |
| Tay-Sachs disease | Hexoseaminidase A and B (*HEXA*, *HEXB*) | AR | Enzyme | 203 |
| SMARD1 | Immunoglobulin μ-binding protein 2 (*IGSMBP2*) | AR | DNA/RNA metabolism | 165 |
| Spinal muscular atrophy | Survival motor neuron (*SMN*) | AR | DNA/RNA metabolism | 153 |
| Lower motor predominant ALS | Mitochondrial isolysinyl tRNA synthetase (*MItRNAS*) | M | Mitochondrial DNA/RNA metabolism | 43 |
| ***Hereditary spastic paraplegia*** | | | | |
| SPG3A | Atlastin (*SPG3A*) | AD | Dynamin-family GTPase; vesicle recycling | 159 |
| SPG17/Silver syndrome | Berardinelli-Seip congenital lipodystrophy type 2 (*BSCL2*) | AD | Transcription factor | 204 |
| SPG13 | Heat shock protein 60 (*HSP60*) | AD | Mitochondrial protein chaperone | 205 |
| SPG10 | Kinesin KIF 5A (*KIF5a*) | AD | Microtubule transport motor | 206 |
| SPG6 | NIPA1 (*NIPA1*) | AD | Possibly membrane protein | 207 |
| SPG4 | Spastin (*SPAST*) | AD | AAA protein, associates with microtubules | 208 |
| Adrenomyeloneuropathy | Adrenoleukodystrophy protein (*ABCD1*) | AR | ATP transporter in peroxisomes | 209 |
| jALS, jHSP | Alsin (*ALS2*) | AR | GEF signalling, vesicle trafficking | 12,13 |
| SPG21 | Maspardin (*SPG21*) | AR | Golgi/vesicle transport and sorting factor | 210 |
| SPG7 | Paraplegin (*SPG7*) | AR | Mitochondrial AAA metalloprotease | 211 |
| SPG20/Troyer syndrome | Spartin (*SPG20*) | AR | Endosomal protein trafficking | 161 |
| Corticospinal predominant ALS | Cytochrome *c* (*COXC*) | M | Mitochondrial electron transport protein | 42 |

AAA, ATPase associated with diverse cellular activities; AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AR, autosomal recessive; CMT, Charcot-Marie-Tooth; GEF, guanine nucleotide exchange factor; HSN, hereditary sensory neuropathy; jALS, juvenile ALS; jHSP, juvenile hereditary spastic paraplegia; M, maternal; SMARD1, spinal muscular atrophy with respiratory distress; SPG, spastic paraplegia; VAMP, vesicle-associated membrane protein.

as the disease progresses[118–120]. Second, retrograde transport is also disrupted in ALS mice[121]. Third, although the molecular basis for this slowing is not fully elaborated, several authors suggest that aggregations of neurofilaments in the proximal axons (spheroids) might physically compromise the transport apparatus[116], at least for anterograde traffic. Neurofilaments have also been incriminated as modulators of axonal transport because they regulate axonal calibre[122]. Diminution of retrograde transport in ALS mice has been attributed to the mislocalization and disruption of dynein function[123].

Human motor neuron disorders other than ALS arise because of perturbations in the structure and function of the motors that mediate axonal transport. Mutations in three different kinesin genes have been implicated in slowly progressive, motor-specific neuropathy, a congenital ocular motor neuropathy and one form of hereditary spastic paraplegia (TABLE 2). In two lines of mice (legs at odd angles and cramping), point mutations in the dynein heavy chain impair motor neuron function and viability[124,125]. Dynein is a major component of the dynein–dynactin retrograde transport motor in neurons and, as noted above, mutations in the p150 subunit of dynactin elicit an unusual form of human lower motor neuron disease. Finally, in mice, disruption of the dynein–dynactin complex by the forced expression of dynamitin, a subunit of dynactin, also produces a late onset motor neuron disease[126]. Among the most intriguing observations from the past two years is that introduction of the dynein mutations that generate either the legs at odd angles[127] or the cramping[128] phenotypes into G93A-SOD1 ALS mice significantly ameliorates the motor neuron disease and the slowing of axonal transport initiated by mutant G93A-SOD1 protein. How this occurs is not understood, although the implication is that some aspect of cytotoxicity derived from mutant SOD1 protein requires dynein-based transport along microtubules, either in the axon or within the soma of the motor neuron.

These diverse observations favour the view that disturbances and attenuation of axonal transport might be a unitary feature of all forms of ALS and potentially a primary pathogenic event in this disease. Challenging this concept is the finding that a genetic mutation (*wlds*) that slows the active process of Wallerian axonal degeneration does not alter the phenotype of G93A-SOD1 mice[129]. However, this negative observation is not entirely surprising, as the *wlds* mutation operates early in development in mice and not at the later ages when motor neurons first degenerate in ALS mice. Two other considerations emphasize the importance of dysfunctional transport in ALS. First, axonal transport is perturbed in mice with other neurodegenerative diseases (for example, Huntington's disease) and *in vitro* by proteins implicated in those diseases (for example, huntingtin and presenilin 1)[130–132]. Second, aberrant vesicular sorting, which is relevant to successful axonal transport, has been implicated both in the *wobbler* mouse (characterized by mutations in a vesicular sorting protein[133] that lead to motor nerve degeneration) and in a human pedigree with frontotemporal dementia[41] (a bifronto-temporal degeneration that can overlap with ALS).

***Excitotoxicity and glutamate transport.*** Another component of neuronal degeneration in many neurodegenerative disorders is excessive glutamate-induced stimulation of postsynaptic glutamate receptors. This activates massive calcium influxes that are potentially detrimental through calcium-activated processes and molecules (for example, proteases, nucleases and lipases). There is considerable evidence in support of this view, such as the observed threefold increase in glutamate levels in the cerebrospinal fluid of patients with ALS[134–136] and the benefits in ALS of the anti-glutamate drug riluzole. EAATs are present at most synapses in the CNS, and transport glutamate from the synaptic space into astrocytes after glutamate release during neurotransmission[137–139]. In most cases (~65%) of SALS, and in transgenic rodent ALS models, there is a profound reduction in the expression and activity of EAAT2 in the cortex and spinal cord[140,141]. One suggestion has been that the loss of EAAT2 arises from splicing errors in the mRNA for EAAT2; indeed, abnormalities such as exon skipping and intron retention have been found in a subset of SALS patients[142]. EAAT2 might be linked to ALS by other mechanisms. In one sporadic ALS case, a germline mutation in *EAAT2* affected *N*-linked glycosylation and glutamate clearance[143,144]. Moreover, in SOD1-linked ALS, oxidative damage inactivated EAAT2 (REF. 140). Similarly, in astrocytes, active CASP3 might also contribute to the reduction of EAAT2 expression and activity by cleaving the transporter at the carboxyl terminus[85]. Because its activity is abnormal in both sporadic human ALS and ALS mice, EAAT2 is a molecular intersection point between these two forms of ALS, and therefore defines one element in a common pathogenetic pathway of ALS.

Glutamate-induced excitotoxicity provides another possible explanation for the selective vulnerability of motor neurons in ALS, because it is predicated on elevations of cytosolic calcium[168] and because, relative to other types of neurons, motor neurons have a diminished capacity to buffer calcium[145]. Additionally, motor neurons express AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/kainate receptors for glutamate, whereas in most neurons glutamate toxicity is mediated by NMDA (*N*-methyl-D-aspartate) receptors. Typically, the GluR2 subunit regulates (and restricts) calcium permeability in AMPA/kainate receptors. Because this subunit is absent in motor neurons, the motor neuronal AMPA/kainate receptor population is unusually calcium permeable and therefore vulnerable to excessive glutamate stimulation[146]. Glutamatergic excitotoxicity can also reflect defective energy metabolism; conceivably, subnormal energy production in ALS motor neurons can sustain glutamate-mediated toxicity without elevations in glutamate.

***Non-neuronal cells affect motor neurons.*** The concept that populations of non-neuronal cells could affect the viability of motor neurons arose originally from the observation that substantial activation of microglial

Table 3 | **Non-neuronal genes that influence motor neuron viability**

| Gene (gene symbol) | Inheritance | Class | Refs |
|---|---|---|---|
| Peripheral myelin protein 22 (*PMP22*) | AD | Myelin/lipid protein | 169 |
| Myelin protein P0 (*P0*) | AD | Myelin/lipid protein | 170 |
| Lipopolysaccharide-induced tumour necrosis factor-α (*LITAF/SIMPLE*) | AD | Protein transport, degradation | 171 |
| Early growth response gene (*EGR2*) | AD | Transcription factor | 172 |
| SOX10 (*SOX10*) | AD | Myelin-specific transcription factor | 212 |
| Ganglioside-induced differentiation-associated protein (*GDAP1*) | AR | Mitochondrial protein | 173 |
| Myotubularin-related protein (*MTMR2*) | AR | Phosphatase | 174 |
| SET binding factor 2 (SBF2) (*MTMR13*) | AR | Myotubularin pseudo-phosphatase | 175 |
| Protein assembly protein (SH3TC2) (*KIAA1985*) | AR | Cytosolic protein | 176 |
| N-myc downstream regulated gene (*NDRG1*) | AR | DNA/RNA metabolism | 164 |
| Periaxin (*PRX*) | AR | Neuronal membrane protein | 177 |
| Connexin 32 (*GJB1*) | XD | Gap junction protein | 178 |
| L1 cell adhesion molecule (*L1CAM*) | AR | Surface protein | 213 |
| Proteolipid protein (*PLP*) | AR | Myelin/lipid protein | 214 |

AD, autosomal dominant; AR, autosomal recessive; XD, X-linked dominant.

and astroglial cells in ALS is one of earliest microscopic manifestations of this disease[147]. Nonetheless, it has been difficult to obtain decisive data to test this hypothesis or to discern whether the activation of astrogliosis or microgliosis is detrimental or beneficial.

Experiments with mice have shown that the specific expression of mutant SOD1 in motor neurons or glia fails to trigger motor neuron degeneration[148,149]. With the caveat that the levels of expression of mutant SOD1 in the targeted cells might have been insufficient to induce a phenotype, these experiments were interpreted to favour the view that motor neuron death in transgenic ALS mice is not cell autonomous. Analyses of chimeric mice with mixed populations of cells expressing either endogenous or transgenic mutant SOD1 were consistent with this view[150]. In these mice, motor neurons expressing transgenic G93A or G37R SOD1 failed to degenerate if they were adjacent to large numbers of supporting cells (such as astrocytes and glia) without the mutant protein. Reciprocally, motor neurons without the transgene demonstrated pathology if surrounded by non-neuronal cells with the mutant *SOD1* transgene. The minimal sets of mutant-expressing cell types required for the development of motor neuron degeneration are still poorly defined. Recent studies with deletable transgenes have documented that expression of the mutant *SOD1* transgene in microglia accelerates the late phase of murine ALS[151]. Parallel studies suggest that expression of the mutant *SOD1* transgene within the macrophage lineage (presumably encompassing microglial cells) is not required for the ALS phenotype[152].

***Other motor neuron disease genes.*** Mutations in more than 50 different human genes are implicated in the pathogenesis of motor neuron cell death[153] (TABLES 2,3; Supplementary information S1 (figure)). Five have been associated with ALS although only two (*SOD1* and *VAPB*) demonstrate the phenotype of ALS. This ensemble of gene defects sheds light on several aspects of ALS.

First, the majority of mutations produce slowly progressive phenotypes, with clinical, pathological and physiological features, suggesting that the burden of the pathology is axonal. That is, most of these disorders might be categorized as axonocentric, with an extremely slow evolution of cell death. By contrast, mutations in two genes (*SOD1* and *VAPB*) trigger an adult onset, fulminant course of rapidly progressive paralysis leading to death, with pathological and physiological characteristics indicating that the cell body is involved early in the disease. These disorders behave as if they are somatocentric, with secondary axonal features.

Second, from the effects of the genetic mutations discussed above, it can be concluded that there are recurring themes in the pathogenesis of these disorders. Fourteen of the genes directly or indirectly implicate aspects of axonal or organelle trafficking. Of these, seven are either cytoskeletal filaments[154,155] or microtubule-based motors[17,157,202,206,208], whereas the other seven are crucial for aspects of vesicle formation, recycling and trafficking (one is involved in GEF signalling[12,13], two are dynamin family GTPases[158,159] and four are central to Golgi and endoplasmic reticular vesicle function[16,160,161,210]). Of note are six genes related to RNA/DNA metabolism[15,153,162,163,165,201] and three HSPs[166,167,205]. The paucity, so far, of other classes of gene is also instructive. So far, only one trophic factor is directly implicated (nerve growth factor and its receptor tyrosine kinase A[196]).

Third, the only defective genes that are not expressed in neurons but nonetheless impair motor neuron viability are expressed in cells that myelinate motor neurons[169–178] (TABLE 3). It has long been established that motor neurons can degenerate if subjected to severe, sustained demyelination. However, apart from the myelin-related genes, none of the other genes in TABLE 2 is expressed exclusively in non-neuronal cells. This is a persuasive argument that non-neuronal cells almost certainly modulate the thresholds or set points for degeneration in motor neurons, although an absolute requirement for the development of mutation-initiated motor neuron disease is the expression of the mutant protein within the motor neuron.

## Conclusion and comments on therapy

Genetic analyses of human motor neuron degeneration have defined diverse molecular pathways in motor neuron cell death. Investigations of mutant SOD1 have illuminated crucial components of the death process including: a propensity for mutant SOD1 to be unstable; a multiplicity of mitochondrial defects that predict cellular energy failure, enhanced glutamate sensitivity and activation of the machinery of programmed cell death;

and a role for non-neuronal cells as modulators of neuron death. What other aspects are likely to be important in ALS? Looking ahead, we anticipate that several themes could emerge: disturbances of vesicular trafficking and axonal transport and further delineation of the ionic basis for excitotoxicity and the mechanisms whereby neurons and support cells compensate for this; more elaborate descriptions of the cellular defences against misfolded proteins; and, in SALS, approaches to detecting extrinsic causative factors (for example, infections and toxins). At present, it seems unlikely that the diverse hypotheses can be combined into a single explanation of ALS. Rather, it is likely that several seemingly disparate factors can trigger motor neuron death as a final common pathway. If there are multiple pathways involved in motor neuron degeneration, there are also multiple targets for therapy. It is beyond the scope of this article to summarize the literature on therapeutic trials in human and rodent ALS (for a review, see REF. 179). However, two lessons are emerging. First, the most effective therapies in ALS mice have delivered the beneficial agents directly to motor neurons. For example, insulin-like growth factor extended survival in the G93A-SOD1 mice when expressed from the type 2 serotype of adeno-associated virus that, in turn, had been carried to the motor neuron by retrograde axonal transport after intramuscular injection[180]. Second, spectacular benefits can be achieved with strategies that inactivate the mutant, disease-causing alleles[181,182]. The most compelling approach to attenuating the diverse, often synergistic downstream pathological processes is to shut off the production of the inciting, upstream protein, whether by RNA interference[181–185], antisense oligonucleotides[186] or some other method. Given contemporary advances in strategies to attenuate gene expression, it is striking that SOD1-mediated ALS, surely the most devastating form of this disease, affecting one-half of all adults in every affected family, might well be the first form of this horrific disease to be treated successfully.


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The authors declare no competing financial interests.

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