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REVIEW ARTICLE

Is SOD1 loss of function involved in amyotrophic lateral sclerosis?

Rachele A. Saccon, Rosie K. A. Bunton-Stasyshyn, Elizabeth M. C. Fisher and Pietro Fratta

Department of Neurodegenerative Disease, Institute of Neurology, University College, London WC1N 3BG, UK

Correspondence to: Elizabeth Fisher
Department of Neurodegenerative Disease,
Institute of Neurology, University College,
London WC1N 3BG, UK
E-mail: elizabeth.fisher@ucl.ac.uk

Correspondence may also be addressed to Pietro Fratta. E-mail: p.fratta@prion.ucl.ac.uk

Mutations in the gene superoxide dismutase 1 (*SOD1*) are causative for familial forms of the neurodegenerative disease amyotrophic lateral sclerosis. When the first *SOD1* mutations were identified they were postulated to give rise to amyotrophic lateral sclerosis through a loss of function mechanism, but experimental data soon showed that the disease arises from a—still unknown—toxic gain of function, and the possibility that loss of function plays a role in amyotrophic lateral sclerosis pathogenesis was abandoned. Although loss of function is not causative for amyotrophic lateral sclerosis, here we re-examine two decades of evidence regarding whether loss of function may play a modifying role in SOD1–amyotrophic lateral sclerosis. From analysing published data from patients with SOD1–amyotrophic lateral sclerosis, we find a marked loss of SOD1 enzyme activity arising from almost all mutations. We continue to examine functional data from all *Sod1* knockout mice and we find obvious detrimental effects within the nervous system with, interestingly, some specificity for the motor system. Here, we bring together historical and recent experimental findings to conclude that there is a possibility that SOD1 loss of function may play a modifying role in amyotrophic lateral sclerosis. This likelihood has implications for some current therapies aimed at knocking down the level of mutant protein in patients with SOD1–amyotrophic lateral sclerosis. Finally, the wide-ranging phenotypes that result from loss of function indicate that *SOD1* gene sequences should be screened in diseases other than amyotrophic lateral sclerosis.

Keywords: amyotrophic lateral sclerosis; motor neuron disease; superoxide dismutase 1; loss of function
Abbreviations: ALS = amyotrophic lateral sclerosis; tg*SOD1* = *SOD1* transgenic mice

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of upper and lower motor neurons. Its clinical course is relentlessly progressive and typically causes death within 3 to 5 years of onset, mostly due to respiratory failure (Haverkamp *et al*., 1995). Similar to other major neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, ALS is typically sporadic, but 5–10% of cases have an autosomal dominant pattern of transmission and are termed 'familial' (Rothstein, 2009). In 1993 the first causative mutations were found within the gene encoding the enzyme, superoxide dismutase 1 (*SOD1*) (Deng *et al*., 1993; Rosen *et al*., 1993). Since then, over 155 *SOD1* mutations have been described (although pathogenicity has not been shown for all of these changes) and these mutations account for up to 20% of

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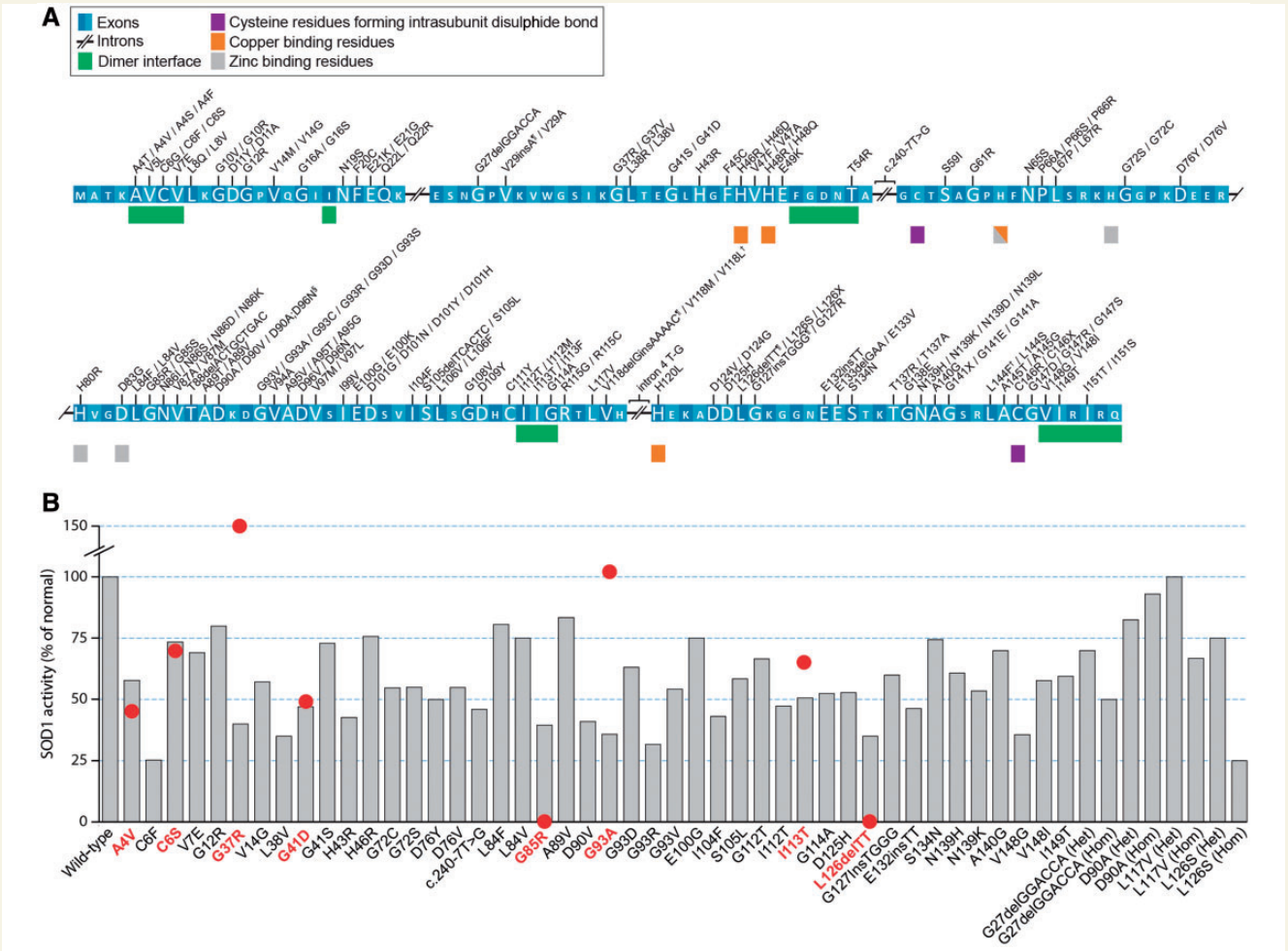

Figure 1 Diagram of human SOD1 mutations, variants and activity in the current literature. The amino acid sequence of SOD1 is shown, with the location of introns (**A**). One hundred and fifty-five SOD1 mutations described in patients with ALS are annotated; data are taken from the ALS online database (ALSoD, http://alsod.iop.kcl.ac.uk, January 2013) and additional literature. Note that only variations that are predicted to affect the amino acid sequence of the protein have been included. Pathogenicity has not been shown for all mutations. Mutations listed on ALSoD, InsAexon2 and E133del are the same as mutations V29insA and E133delGAA, respectively, and so have not been annotated separately. Similarly, we believe the mutation D125TT to be L126delTT and mutation E133insTT to be E132inTT. Information about highlighted structural elements was from Wang *et al*. (2006). Additional references are Pramatarova *et al*. (1995) and Kobayashi *et al*. (2012). [†]Locations where two nucleotide changes results in the same amino acid substitution; [¶]Mutations which result in a frameshift and premature stop codon. (**B**) Diagram of human SOD1 mutations and overall enzyme activity measured in red blood cells, fibroblast and lymphoblast cell lines. Measurements from patients carrying 48 SOD1-familial ALS mutations between 1993 and December 2012; original references are cited in Supplementary Table 1. All measures fall below 100% normal activity. Three mutations found in homozygous individuals are shown on the right hand side of the figure. Red circles show measures of intrinsic activity where these are known. We note that all mutations shown here are familial, not sporadic, and have supporting data indicating they are ALS causative (Supplementary Table 1). Where more than one publication shows overall activity for an individual mutation the value from the report with the highest sample size has been plotted. Refer to supporting references for details. Het = heterozygous; Hom = homozygous.

familial ALS cases (Fig. 1) and 3% of sporadic ALS cases (Pasinelli and Brown, 2006; Acevedo-Arozena *et al*., 2011; Andersen and Al Chalabi, 2011).

SOD1 is ubiquitously expressed and highly conserved across species (Fridovich, 1995). The gene is composed of five exons encoding a 153 amino acid metalloenzyme, also referred to as Cu/Zn superoxide dismutase. The protein localizes to the cytoplasm, nucleus, lysosomes and intermembrane space of mitochondria (Chang *et al*., 1988; Keller *et al*., 1991; Crapo *et al*., 1992; Sturtz *et al*., 2001). It binds copper and zinc ions and forms a homodimer whose main known function is as a dismutase removing dangerous superoxide radicals by metabolizing them to molecular oxygen and hydrogen peroxide, thus providing a defence against oxygen toxicity. Recently, SOD1 has been found to be critical for repressing respiration and directing energy metabolism through integrating responses to O_2, glucose and

superoxide levels (through casein kinase signalling); this role is independent of its function in oxidative stress (Reddi and Culotta, 2013). Other described functions are nitration of proteins (Beckman *et al*., 1993), copper buffering (Culotta *et al*., 1997), phosphate activation (Wang *et al*., 1996), zinc homeostasis (Wei *et al*., 2001), thiol oxidation, and immunomodulation—modulation of SOD1 activity may differentially affect the NO-dependent microbicidal activity and release of cytokines by activated macrophages (Marikovsky *et al*., 2003). Further, SOD1 is produced at high levels that have not yet been explained by known functions, therefore, it may well play other roles both neuron-specific and generally (Reddi and Culotta, 2013).

The finding of loss of dismutase activity in patients with ALS and the distribution of ALS-causative mutations spread throughout the *SOD1* gene initially suggested loss of function as a mechanism (Deng *et al*., 1993; Rosen *et al*., 1993). However, evidence for a gain of function mechanism was quick to follow from analysis of mutant *SOD1* transgenic (tg*SOD1*) mouse models. The first of these, tg$SOD1^{G93A}$, carries an ALS-causative point mutation resulting in a glycine to alanine substitution at residue 93 (Gurney *et al*., 1994). Currently there are more than 12 different published human mutant SOD1 transgenic strains (Joyce *et al*., 2011) and most of them (including tg$SOD1^{G93A}$) have increased dismutase activity because they greatly overexpress transgenic mutant human SOD1 in addition to endogenous mouse SOD1 and they develop a progressive adult-onset motor phenotype, accompanied by a striking loss of lower motor neurons.

Thus a loss of function mechanism became less favoured compared with gain of function because: (i) in humans, a lack of correlation was found between SOD1 dismutase activity and aggressiveness of clinical phenotypes (Ratovitski *et al*., 1999); (ii) in mice, a lack of overt ALS-like phenotype was found in *Sod1* null ($Sod1^{-/-}$) animals, the first of which was published by Reaume *et al*. (1996); whereas (iii) transgenic mouse models over-expressing mutant human SOD1 have increased SOD1 activity and a loss of motor neurons that models human ALS.

The death knell for loss of function came in a seminal study published by the Cleveland laboratory (Bruijn *et al*., 1998) who analysed survival time in mice carrying a mutant *SOD1* transgene (tg$SOD1^{G85R}$) on a normal mouse background (i.e. with two copies of the endogenous mouse *Sod1* gene) compared with the same transgene on a $Sod1^{-/-}$ background. They found no change in survival of the mice, thus concluding that survival was entirely due to a gain of function mechanism, and independent of mouse SOD1 loss of function. These findings essentially ended the debate for a role of loss of function as a contributor to SOD1-familial ALS (Bruijn *et al*., 1998).

SOD1 mutations remained the only known cause of 'classical' ALS until causative mutations in the gene *TARDBP* were found (Sreedharan *et al*., 2008), and therefore have been studied extensively in a variety of animal and cellular models (Ilieva *et al*., 2009). The causative gain of function is indisputable and several mechanisms by which this occurs have been proposed and comprehensively reviewed (Turner and Talbot, 2008; Ilieva *et al*., 2009; Rothstein, 2009). However, in recent years a number of laboratories have further investigated *Sod1* knockout mice, examining their neuromuscular involvement and non-neurological features. Data from these investigations and recent experiments using transgenic *SOD1* overexpressing mice have pointed to the possibility of a modifying role played by loss of dismutase activity on familial ALS disease course.

Here we review what is known about SOD1 loss of function and the evidence to suggest it may play a role in ALS pathogenesis after all, possibly through increased susceptibility to neurodegeneration. It is important and timely to consider these data because they are relevant to current therapeutic strategies to reduce the level of mutant familial ALS-SOD1 in heterozygous individuals. Such strategies could provide badly needed approaches to ameliorating the ALS phenotype, but clearly SOD1 loss has effects on both neuronal and non-nervous system tissues. SOD1 loss of function data also strongly suggest that SOD1 should be screened in disorders other than ALS.

SOD1 dismutase activity is greatly reduced in patients with SOD1-familial amyotrophic lateral sclerosis

Dismutase activity is the best characterized function of SOD1. Two other dismutases, encoded by separate genes, have been identified in mammals: SOD2 (Mn-SOD), which has manganese (Mn) as a cofactor and localizes to the mitochondrial matrix (Fridovich, 1986; Zelko *et al*., 2002) and SOD3 (Fe-SOD), which exists as a homotetramer and is mainly extracellular (Marklund *et al*., 1982). The existence of three enzymes with dismutase activity may complicate measures of SOD1 activity alone.

Box 1 Methods of measuring SOD1 dismutase activity

Activity assay:	A sample is collected from the tissue of interest, such as red blood cells. Xanthine–xanthine oxidase is added to the sample to generate superoxide anions (O_2^-), and then a chromagen is used as an indicator of O_2^- production. In the presence of SOD, O_2^- concentrations are reduced, resulting in decreased colorimetric signal. However, all three SOD isoforms contribute to the activity measured, and so SOD1 activity is obtained indirectly by subtracting SOD2 and SOD3 activity from total SOD activity. This is achieved by running a parallel assay with the addition of potassium cyanide which preferentially inhibits SOD1 (Roe *et al*., 1988).
Gel assay:	Proteins from the tissue of interest are separated by electrophoresis in a native gel which is subsequently stained using a solution of nitro blue tetrazolium and riboflavin. Riboflavin is a source of O_2^- when exposed to light. The superoxide anions interact with nitro blue tetrazolium, reducing the yellow tetrazolium within the gel to a blue precipitate. This reduction reaction stains the gel blue; however, SOD inhibits this reaction, resulting in colourless bands where SOD is present. As the intensity of these bands is relative to the amount of SOD present, quantification can be inferred by measuring the intensity of the bands at the correct molecular weight using digital software (Weydert and Cullen, 2010).

In patients with ALS, SOD1 activity has most commonly been measured using two methods, generally either (i) the 'activity assay' which measures total dismutase activity of all three enzymes and then subtracts SOD2 and SOD3 activity, to leave only SOD1 activity; or (ii) the 'gel assay' in which the three enzymes are separated by electrophoresis and then dismutase activity is measured within the band of the correct size for the SOD1 dimer (Box 1).

Intrinsic and overall SOD1 activity

The dismutase activity of SOD1 can be measured in two ways depending on whether the focus is on (i) the 'intrinsic' SOD1 activity, which reflects the enzymatic efficiency of the protein and is obtained by measuring the activity of recombinant SOD1 protein normalized to its quantity; or (ii) the 'overall' activity within a tissue sample, which may be affected by various factors in the cellular environment (as described below), and is obtained by normalizing dismutase activity to the quantity of tissue. This last measure has generally been used with patients with familial ALS, and is a reflection of the amount of activity present in a biological context.

Intrinsic activity influences the overall activity, but is only one of the determinants; the others being any factor that affects the quantity, biological availability and functionality of SOD1. Amongst these are *SOD1* messenger RNA half-life, SOD1 protein half-life, correct folding of SOD1, Cu_2^+-loading of SOD1 and other post-translational modifications (Wilcox *et al*., 2009). In general, the 'overall' activity is an unbiased measure that takes into account known and unknown influences on SOD1 enzyme activity. Measurements are generally expressed relative to normal control samples.

Overall SOD1 activity is reduced in human amyotrophic lateral sclerosis samples

Intrinsic SOD1 activity has been measured in at least eight mutant proteins, giving diverse results ranging from 0–150% of human wild-type SOD1 activity. Correlation between these values and clinical aspects of the disease was assessed and did not show significant links (Borchelt *et al*., 1994; Ratovitski *et al*., 1999).

However, overall SOD1 activity has most commonly been measured in red blood cells, fibroblasts and lymphoblastoid cell lines derived from patients carrying at least 48 different mutations, and has proved to be more homogeneous than measures of intrinsic activity. We have searched reports of SOD1 activity from 1993 to 2012 in patients with SOD1–familial ALS and found that the majority of 48 tested mutations all have a reduction of overall activity. The average loss of activity is notable and averages 58% (±17, SD) of normal values (Fig. 1). These results are of strikingly consistency given the variability arising from different laboratories performing the measurements and the naturally occurring variation in activity documented in blood samples (de Lustig *et al*., 1993; Borchelt *et al*., 1994; Robberecht *et al*., 1994).

Overall SOD1 activity is normal or only slightly reduced in two mutations: the D90A in both homozygous and heterozygous patients, and the L117V in heterozygotes; although measurement from a homozygous patient showed a reduction of 67% SOD1 activity compared with control subjects (Andersen *et al*., 1995; Synofzik *et al*., 2012). Both these mutations are atypical, for example (i) SOD1 misfolding is not detected in cells derived from patients carrying these mutations (see below for association between SOD1 misfolding and SOD1 activity); (ii) the disease allele is homozygous in the majority of patients with D90A ALS and in one of four reported patients with L117V ALS; (iii) penetrance in heterozygotes is low; and (iv) disease progression is unusually slow (Synofzik *et al*., 2012). Possibly these mutants have a slightly increased propensity to aggregate that is sufficient to start the disease process, although with reduced frequency, in the CNS, but is not enough to determine misfolding and loss of dismutase activity in the periphery.

Of note is the non-correspondence between the overall patient activity and the intrinsic activity; for example, the $SOD1^{G37R}$ mutant has 150% intrinsic activity but only 40% overall activity compared with normal control subjects. Given that the intrinsic activity is only one of the determinants of the overall activity, other factors, such as the stability of mutant SOD1 protein, have been investigated to try to account for this loss of activity.

Different mutant SOD1 proteins have been shown to have a variable half-life, but this is consistently reduced compared to the wild-type form (Sato *et al*., 2005). Calculations combining the measurement of intrinsic activity and half-life of six SOD1-familial ALS mutant proteins predicted that the overall activity would have been only 50% of normal levels (Borchelt *et al*., 1994). Other studies and Fig. 1 are in accord with this finding (Deng *et al*., 1993; Birve *et al*., 2010). Further, most measures of SOD1 activity from patients with ALS have been taken in red blood cells, and we note that these cells have no active protein synthesis and are on average 60 days old, making the system particularly responsive to detecting protein half-life changes (Broom *et al*., 2008). Nevertheless, when tested in post-mortem brain and spinal cord, SOD1 activity was found to be reduced to levels similar to those measured in red blood cells (Bowling *et al*., 1993; Rosen *et al*., 1994; Watanabe *et al*., 1997; Browne *et al*., 1998; Jonsson *et al*., 2004), and when activity in red blood cells and CNS was compared in the same subset of patients, results were strikingly concordant (Rosen *et al*., 1994).

Is SOD1 dismutase activity reduction exacerbated in motor neurons?

SOD1 activity has not been specifically measured in motor neurons and other affected cell types from SOD1–familial ALS post-mortem material, owing to technical difficulties in conducting these assays on limited micro-dissected material. The level of

activity reduction in these cell types is therefore unknown; however, there is evidence that the dismutase activity reduction may be enhanced in motor neurons.

RNA

SOD1 messenger RNA was shown to form tissue-specific complexes with ribonucleoproteins from brain and spinal cord and these interactions prolong its half-life in these tissues. However, complex formation appears to be impaired when *SOD1* messenger RNA carries ALS-causing mutations, therefore potentially reducing the half-life of mutant *SOD1* messenger RNA preferentially in CNS of patients with SOD1-familial ALS (Ge *et al*., 2006).

Aggregation

Indirect evidence indicates that SOD1 aggregation reduces dismutase activity. For example, data from cell experiments in which $SOD1^{G93A}$ and amyloid-β were co-expressed, suggesting that aggregation results in reduction of SOD1 activity (Yoon *et al*., 2009). Further, two transgenic mouse lines over-expressing human wild-type-SOD1 at a high level and at 2-fold this level, were analysed for aggregation and SOD1 activity. In liver and muscle samples, aggregation was not found to be present, SOD1 protein levels were increased, as expected, 2-fold and SOD1 activity increased accordingly. However, in spinal cord and brain, where SOD1 aggregation was clearly found, protein levels had a similar increase as in muscle and liver, but importantly SOD1 activity did not show an increase, strongly suggesting a link between protein aggregation and activity (Graffmo *et al*., 2013). Furthermore, even if SOD1 retains enzymatic activity in aggregates, it may not accomplish the same functions as when correctly targeted to the appropriate cell compartments. SOD1 misfolding and aggregation are a hallmark of SOD1-familial ALS and have been extensively documented in SOD1-familial ALS (Jonsson *et al*., 2008) and wild-type SOD1 has also been shown to be present in spinal cord aggregates of both patients with familial ALS (Jonsson *et al*., 2004) and SOD1 mouse models (Deng *et al*., 2006; Wang *et al*., 2009*a*; Prudencio *et al*., 2010), thus making a dominant negative loss of function plausible. The recent finding of SOD1 aggregation in cases with sporadic ALS (Bosco *et al*., 2010; Forsberg *et al*., 2010) makes this mechanism potentially relevant also to sporadic disease.

Oxidative stress

Lastly, oxidative stress induces SOD1 to monomerize as an intermediate step to aggregate formation and it is known that SOD1 does not have dismutase activity in this form (Khare *et al*., 2004; Rakhit *et al*., 2004; Ezzi *et al*., 2007; Wilcox *et al*., 2009). Motor neurons are known to be particularly susceptible to oxidative stress (Barber and Shaw, 2010) making this process potentially more pronounced in these cell types.

The tissue-specific changes in *SOD1* messenger RNA half-life and the effect of SOD1 aggregation and monomerization on dismutase activity, raise the possibility that SOD1 activity in the affected neurons may be lower than that measured from blood. How SOD1 activity in blood relates to that in spinal cord motor neurons is a critical issue that remains to be addressed.

In summary, SOD1 overall activity is consistently reduced in blood samples of patients with SOD1–familial ALS, likely owing to changes in protein activity and alterations in mutant protein half-life. Further, there is an additional possible tissue-specific dismutase activity reduction in neurons.

Sod1$^{-/-}$ mice have neuromuscular, neuronal and extra-neuronal phenotypes

An approach to elucidating the effect of SOD1 loss of function is to assess the phenotype of *Sod1* null (*Sod1*$^{-/-}$) mice. Homozygous *Sod1* null mice have been used to analyse the role of SOD1 in ALS, and for other purposes such as studying oxide radical-mediated toxicity in reproduction and development (Huang *et al*., 1997; Matzuk *et al*., 1998). To date five *Sod1* knockout mouse lines have been published: *Sod1*^{tm1Cpe} (Reaume *et al*., 1996); *Sod1*^{tm1Cje} (Huang *et al*., 1997); *Sod1*^{tm1Leb} (Matzuk *et al*., 1998); *Sod1*^{tm1Ysh} (Ho *et al*., 1998); and *Sod1*^{tm1Dkd} (Yoshida *et al*., 2000). All were obtained by targeted deletion of different regions of the *Sod1* gene, ranging from a single exon to the entire genomic sequence. For all five lines, no SOD1 protein is detectable in homozygous null mice and SOD1 activity is absent or very low [which may represent the background of the enzyme assay or might be caused by an endogenous superoxide dismutase activity supplied by an alternative scavenging enzyme (Reaume *et al*., 1996)].

Deletion of different portions of the same gene may result in different phenotypes, but the five *Sod1* knockout strains have been compared in a number of studies and are strikingly similar (Huang *et al*., 1997; Kondo *et al*., 1997; Kostrominova, 2010). For example, skeletal muscles of three different *Sod1* null strains were compared and all developed accelerated age-related muscle denervation (Kostrominova, 2010). Genetic background may also influence phenotypes, however the majority of the studies discussed here, have been carried out on *Sod1*$^{-/-}$ mice on a congenic C57BL/6 background, making results from different laboratories comparable.

When *Sod1*$^{-/-}$ mice were first generated, a key issue was whether they developed motor neuron degeneration. The first analysis, from Reaume *et al*. (1996), found no reduction in motor neuron number at 4 months of age, but an increase in small neurons and astrocytes in spinal cord ventral horns. Subsequent studies confirmed the lack of motor neuron loss at 6, 9 and 17 months (Flood *et al*., 1999) and lack of vacuolation or chromatolysis, key features of ALS, at 4 and 18 months (Fischer *et al*., 2012). Microgliosis and ubiquitinated protein accumulation in motor neurons were ruled out, but mild astrocytosis was found at 4 and 18 months (Fischer *et al*., 2012). Evaluation of lumbar ventral roots confirmed no loss in motor neuron number, but interestingly axonal diameter was reduced at 6 and 19 months and evidence of degenerating and regenerating axons was seen in the ventral L3 root at the latter time point (Flood *et al*., 1999),

although another study did not see these results when analysing the L4 root (Fischer *et al*., 2012).

Although *Sod1* null mice do not develop overt motor neuron degeneration, there is now a considerable literature, summarized below and described in more detail in the Supplementary material, showing that compared with wild-type controls, these mice have a wide range of phenotypes, including several relevant to ALS, such as a slowly progressive motor deficit that manifests from early adulthood and likely involves defects in large motor axons.

Sod1 null mice develop an adult-onset progressive motor axonopathy

Behavioural data

Sod1 null animals appear indistinguishable from littermates at birth through to weaning (Reaume *et al*., 1996). Weight reduces with age compared with wild-type littermates (Jang *et al*., 2010; Larkin *et al*., 2011) and voluntary wheel running diminishes, which is a sensitive test for early locomotor defects (Muller *et al*., 2006). At 9 months of age Rotarod performance and stride-length worsen (Flood *et al*., 1999; Muller *et al*., 2006), although spontaneous locomotion is normal, thus changes in motivation are not likely to contribute to these results (Flood *et al*., 1999; Muller *et al*., 2006). At 1 year of age, $Sod1^{-/-}$ mice also have a deficit in grip-strength, and tremors (Fischer and Glass, 2010)—hallmarks of neuromuscular disorders in mice (Muller *et al*., 2006).

Neurophysiological data

Direct neurophysiological measurement of the response to stimulation of nerve and muscle eliminates behavioural/motivational variability from the outcome. Such studies showed *Sod1* null mice have a reduction in muscle strength suggesting a progressive deficit in innervation (Larkin *et al*., 2011). Motor unit number is reduced at 3 months, with progressive loss with age (Shefner *et al*., 1999). Complex repetitive discharges found on needle examination indicate a deficit in the terminal part of the motor axon (Shefner *et al*., 1999). Measures of nerve conduction velocity and latency analysis on sensory and mixed nerves, show a reduction only where a motor component is present, compatible with a deficit in the largest motor axons (Flood *et al*., 1999). Thus neurophysiological investigations show clear muscle denervation and deficits in motor axons and functional motor units in *Sod1* null mice.

Axonal damage and early involvement of neuromuscular junctions

Denervation in $Sod1^{-/-}$ mice has been documented by neuromuscular junction analysis of both fast and slow twitch muscles (Flood *et al*., 1999; Fischer *et al*., 2011, 2012; Larkin *et al*., 2011). Denervation progresses with age, maintaining a more aggressive pattern in fast-twitch rather than slow-twitch muscles (Jang *et al*., 2010; Fischer *et al*., 2012). Thus SOD1 is required for maintenance of motor axons and their terminals (Fischer *et al*. (2011) and without this protein, fast-twitch motor units are lost preferentially, as is also observed in patients with ALS and mouse models of familial ALS (Dengler *et al*., 1990; Pun *et al*., 2006). The involvement of neuromuscular junctions as an early pathological target has been extensively documented in mouse models of SOD1–familial ALS (Murray *et al*., 2010) and was shown to precede motor neuron cell body loss in early disease in a patient with ALS (Fischer *et al*., 2004).

Muscle pathology is secondary to denervation

Muscle mass is progressively lost (Muller *et al*., 2006; Jang *et al*., 2010; Larkin *et al*., 2011) but has not been reported for organs such as liver, heart or kidney (Muller *et al*., 2006). Angular muscle fibres, indicating denervation, are present by 2 months of age (Flood *et al*., 1999) and by later time points a massive reduction in fibre number occurs, preferentially affecting type 2b fibres (fast glycolytic type innervated by large motor neurons), along with an increase in angular fibres (Reaume *et al*., 1996; Larkin *et al*., 2011). This muscle profile also occurs in mouse models of familial ALS–SOD1 (Kennel *et al*., 1996; Frey *et al*., 2000) and is typical of the neurogenic changes that initially affect larger motor neurons, suggesting that muscle pathology is secondary to axonal events.

Confirmation of muscle pathology being secondary to axonal damage and denervation was obtained by crossing $Sod1^{-/-}$ mice with transgenic mice expressing *Sod1* in the CNS but not in muscle. In double mutant progeny, muscle pathology was fully rescued despite the absence of SOD1 in muscle (Flood *et al*., 1999). Further, in $Sod1^{-/-}$ mice, measurements of steady state redox potential of glutathione (which is routinely used as indicator of the intracellular redox state) in tibial nerve and gastrocnemius muscle showed a selective involvement of the nerve at 4 months, again indicating the primary involvement of the axon (Fischer *et al*., 2012). Thus muscle changes in $Sod1^{-/-}$ mice are secondary to denervation; they are non-specific and also present in muscle biopsies from patients with ALS (Baloh *et al*., 2007*a*).

$Sod1^{-/-}$ motor neurons show increased vulnerability to stress

SOD1 activity is important for motor neuron survival after injury as shown by facial axotomy of *Sod1* null mice which resulted in a significant increase in motor neuron loss compared with wild-type controls. This is interesting when considering the potential role for injury and trauma in ALS (Pupillo *et al*., 2012; Yip and Malaspina, 2012).

Selective susceptibility to damage of the motor system

An important feature of ALS is the selective involvement of motor neurons and their related circuits, and indeed the phenotypes induced by lack of SOD1 in mice preferentially affect motor neurons. $Sod1^{-/-}$ mice have no significant deficits in somatosensory behaviour (Flood *et al*., 1999). Further, neurophysiology testing showed preferential motor involvement in $Sod1^{-/-}$ mice and, on histopathological examination, L3 dorsal roots at 19 months are normal in contrast to the ventral roots, which have signs of degeneration/regeneration (Flood *et al*., 1999). Finally, analysis of *Sod1* null epidermal nerves, which are the most distal tracts of the sensory axons, showed no abnormality, in contrast to their severely affected motor counterparts, the neuromuscular junctions (Fischer *et al*., 2012).

Loss of SOD1 affects mitochondrial function

Sod1$^{-/-}$ mice lack superoxide scavenging function in the cytosol and mitochondrial intermembrane space, which contain O_2^- generated by complex III (Muller *et al*., 2004). *Sod1* null mitochondria release significantly increased amounts of O_2^- and therefore increased oxidative stress in these compartments was hypothesized to account for the neuromuscular phenotype of the mice (Jang *et al*., 2010). Fischer *et al*. (2011) demonstrated that mitochondrial density is reduced in *Sod1*$^{-/-}$ axons and, remarkably, they reversed this loss and the neuromuscular phenotype, by replacing SOD1 selectively in the mitochondrial intermembrane space of these mice.

Mitochondrial dysfunction is associated with ALS (Faes and Callewaert, 2011) and mitochondria are important for distal axonal maintenance (Baloh *et al*., 2007*b*; Cassereau *et al*., 2011). Furthermore, mitochondrial transport abnormalities are described in tg*SOD1*-ALS mouse models (De Vos *et al*., 2007). Also, abnormal mitochondrial accumulations have been described in lower motor neurons and proximal axons from patients with ALS, post-mortem (Sasaki *et al*., 2009). Thus it seems likely that damage to mitochondria through raised levels of free radicals may have a significant effect on distal axons of motor neurons.

In summary *Sod1*$^{-/-}$ mice appear normal up to the age of weaning, after which they develop a slowly progressive motor-neuronopathy that involves primarily the motor neuron axons and neuromuscular junctions and is accompanied by significant secondary denervation pathology in muscles (Supplementary Fig. 1). Sensory involvement is negligible. Although no motor neuron loss has been documented, these are more vulnerable to damage in *Sod1*$^{-/-}$ mice.

Other neuronal and extra-neuronal *Sod1* null phenotypes

In addition to deficits in the motor system, *Sod1* null mice develop a range of other disorders including: progressive neuronal hearing loss; progressive retinal degeneration; greatly increased susceptibility to cerebral ischaemia and brain trauma. Most importantly for studies of ALS, these mice have an increased susceptibility to neurodegeneration, for example, when crossed to a mouse model of Alzheimer's disease (Supplementary Box 1).

Extra neuronal phenotypes are also striking, in particular the well-known susceptibility to hepatocellular carcinoma, a feature that also manifests in the human population; there is a positive correlation between SOD1 activity and postoperative hepatocellular carcinoma survival time, as well as low levels of SOD1 and severity of hepatocellular carcinoma (Elchuri *et al*., 2005; Takahashi *et al*., 2002; Casaril *et al*., 1994; Liaw *et al*., 1997; Lin *et al*., 2001). Other non-neuronal features include impaired endothelial-dependent relaxation, thinning of the skin, osteoporosis and female infertility (Supplementary Box 1).

These remarkably wide-ranging phenotypes are perhaps not surprising in an animal that is missing such an important enzyme, but the tissue specificity partly points to effects in tissues with a high production of free radicals, such as the nervous system and liver. Further, the progressive nature of most of these phenotypes is striking and may be relevant to the mechanism of SOD1–familial ALS, both in terms of deficits increasing with age and in terms of targeting of many of the deficits to neuronal tissues.

A SOD1 activity of 50% is not sufficient for normal neuronal function

Sod1$^{+/-}$ mice have abnormal phenotypes including within the motor system

Sod1 null mice are invaluable for investigating *in vivo* consequences of SOD1 loss of function, and may provide clues for the effects of reduced enzyme activity in ALS. However, the 100% loss of enzyme activity is a different setting from the average 57% reduction in patients with ALS. Thus, although *Sod1* null mice clearly indicate a susceptibility of specific tissues to the effects of a loss of SOD1 function, any discussion in the context of ALS must look at phenotypes that arise in *Sod1*$^{+/-}$ animals (Supplementary Fig. 1) that retain 50% SOD1 activity (Reaume *et al*., 1996) and so mimic the physiological levels described in patients with SOD1–familial ALS. Although *Sod1*$^{+/-}$ mice clearly do not develop an ALS-like syndrome, a wide range of studies show that *Sod1*$^{+/-}$ mice have abnormal phenotypes involving progressive cellular damage and deficits in reaction to injury and toxic stimuli. Here we consider how these may have implications for human ALS.

Sod1$^{+/-}$ motor neurons are more susceptible to cell death after axon injury

Sod1$^{+/-}$ mice suffer significantly more motor neuron loss in response to facial nerve axotomy than wild-type mice. This result is intermediate between *Sod1*$^{-/-}$ and control mice, suggesting a dose dependence of this effect and demonstrating that 50% SOD1 activity is not sufficient for a normal function of motor neurons in response to injury (Reaume *et al*., 1996).

Facial nerve axotomy was also performed on copper chaperone for SOD1 null mice (*Ccs*$^{-/-}$), that retain only 20% of SOD1 activity, owing to the lack of this crucial protein for delivery of copper to SOD1 (Box 2). Motor neuron survival was significantly reduced in *Ccs*$^{-/-}$ mice (Subramaniam *et al*., 2002). This result,

BOX 2 *Ccs*$^{-/-}$ null mice model SOD1 partial loss of activity

Another mouse model that is relevant to studying the effects of reduced SOD1 activity is the 'copper chaperone for SOD1' (*Ccs*) null mouse. Copper chaperones shuttle copper, which is toxic for cells in its free form, to intracellular target proteins. CCS delivers copper to SOD1 by direct protein–protein interaction and is required for full activation of SOD1 (Culotta *et al*., 1997, 1999). Mice lacking CCS (*Ccs*$^{-/-}$) were generated by gene targeting and retain only 20% of normal SOD1 activity; CCS-independent copper loading into SOD1 probably accounts for the remaining activity. Although the dismutase activity is impaired, there is no difference in the levels of SOD1 protein among wild-type, *Ccs*$^{+/-}$ and *Ccs*$^{-/-}$ littermates (Wong *et al*., 2000).

and the similar result from *Sod1*$^{+/-}$ mice, is important in light of the potential role for injury and trauma as a trigger in ALS pathogenesis (Pupillo *et al*., 2012; Yip and Malaspina, 2012).

Spontaneous denervation, motor neuron sensitivity and reduction in mitochondrial numbers are not significant in *Sod1*$^{+/-}$ mice but all show trends

Although *Sod1*$^{+/-}$ mice have been less studied than null animals, there have been comprehensive investigations of denervation in these mice (Fischer *et al*., 2011, 2012). At 18 months, 80% of tibialis anterior muscle (neuromuscular junctions) were innervated in *Sod1*$^{+/-}$ compared with 92% in controls (Fischer *et al*., 2011). This result did not reach statistical significance, but this trend was repeated in a following study (Fischer *et al*., 2012). Currently, there is no evidence for spontaneous denervation in *Sod1*$^{+/-}$ mice. Experiments were conducted up to the time-point of 18 months, the maximum limit when investigating *Sod1*$^{-/-}$ mice—which develop and die of liver cancer at this stage—but could be extremely informative at later time-points for the *Sod1*$^{+/-}$ mice. Whether increased sample size or a later time-point would show a significant result, remains to be determined.

Glutamate toxicity is enhanced in *Sod1*$^{+/-}$ mice

Glutamate toxicity is implicated in disease in patients with ALS and in animal models (Ilieva *et al*., 2009). The role of SOD1 in neuronal sensitivity to glutamate toxicity was assessed *in vivo* by intrastriatal injection of *N*-methyl-D-aspartic acid and kainite glutamate receptor agonists. *Sod1*$^{+/-}$ mice were more susceptible to the neurotoxic effects of both stimuli and had reduced glutamic acid decarboxylase and choline acetyltransferase activities compared with controls (Schwartz *et al*., 1998). Thus SOD1 partial loss of function could play a role in facilitating damage from glutamate toxicity, which may have relevance to ALS.

Increased susceptibility to cerebral ischaemia in *Sod1*$^{+/-}$ mice

Sod1$^{+/-}$ mice have decreased survival after induced focal cerebral ischaemia, along with increased early blood–brain barrier disruption and increased infarct volume causing brain swelling. Apoptotic neuronal death is also increased demonstrating enhanced ischaemia–reperfusion injury (Kondo *et al*., 1997). Intriguingly, an important mechanism involved in ischaemia–reperfusion injury is glutamate excitotoxicity, which as discussed above, is postulated to play a role in ALS pathogenesis (Beal, 1992).

We note that blood–brain barrier alterations are found in tg*SOD1*-ALS mouse models and that indirect evidence of disruption, such as increased cerebrospinal fluid (CSF) albumin/plasma albumin ratios, has been documented in patients with ALS (Leonardi *et al*., 1984; Apostolski *et al*., 1991).

Increased memory deficits and plaque formation in an Alzheimer's disease model on a *Sod1*$^{+/-}$ background

Overexpression in mice of the *APP* gene carrying the Swedish mutation causes behavioural deficits and plaque formation and thus models Alzheimer's disease (Bodendorf *et al*., 2002). When this mutation is expressed on a *Sod1*$^{+/-}$ background, it results in increased deficits in behavioural tests used to assess memory and in increased senile plaque formation, thus showing that lack of 50% of SOD1 activity does indeed increase the development of a neurodegenerative phenotype *in vivo*.

Ganglion neuron loss is increased with ageing in *Sod1*$^{+/-}$ mice

A 50% reduction in SOD1 activity results in reduced neuronal survival *in vivo* with respect to ganglion cell density, although this does not cause an apparent hearing deficit (Keithley *et al*., 2005).

DNA methylation is reduced in *Sod1*$^{+/-}$ mice

The effects of reduced mouse SOD1 activity could be relevant to ALS because DNA methyltransferases, the enzymes involved in DNA methylation, and 5-methylcytosine, the end-product of DNA methylation, were found to be upregulated in human ALS, suggesting that aberrant regulation of DNA methylation is part of the pathobiology of ALS (Chestnut *et al*., 2011). DNA methylation was significantly reduced at 2 months of age in *Sod1*$^{+/-}$ mice, although this study focused on prostate tissue (Bhusari *et al*., 2010).

Sod1$^{+/-}$ mice exhibit a contractile vascular phenotype with ageing

High levels of superoxide play a major role in contractile vascular dysfunction and loss of a single copy of *Sod1* is enough to increase vascular superoxide levels and produce vascular contractile dysfunction with ageing (Didion *et al*., 2006).

Loss of mouse SOD1 activity to 50% of normal levels does not cause death of motor neurons but may be relevant to human SOD1–familial amyotrophic lateral sclerosis

Sod1$^{+/-}$ mice show an increased loss of specific neuronal subtypes with ageing and an increased susceptibility to injury and toxic stimuli. These results are relevant to SOD1-familial ALS given the similarity of enzyme activity levels between patients with SOD1-familial ALS and *Sod1*$^{+/-}$ models. The vulnerability shown in motor neurons after injury, the susceptibility of neurons to glutamate toxicity, and the blood–brain barrier alterations seen in these mice are significant elements since they are mechanisms and alterations postulated to be involved in ALS pathogenesis.

Overall *Sod1*$^{-/-}$ and *Sod1*$^{+/-}$ animals do not recapitulate mouse ALS, but they have a wide range of phenotypes that are both related to ALS directly (for example, denervation, increased susceptibility to glutamate toxicity, increased susceptibility to axonal damage) and more generally to neuronal degeneration (for example, loss of ganglion and retinal cells) and therefore this raises the question of a contribution of *SOD1* loss of function to disease. A further point to consider is that although several transgenic mice carrying *SOD1* mutations have motor neuron degeneration and characteristics of human ALS, other mouse strains with well-characterized pathogenic mutations in different 'ALS genes' (for example, *TARDBP*) have phenotypes less clearly reminiscent of the human disease (Joyce *et al*., 2011). Thus it remains debatable as to how a mouse–ALS syndrome might manifest, and so considering all phenotypes that develop in the CNS of these

models is essential for understanding how ALS mutations cause disease.

SOD1 activity and its influence on SOD1–familial amyotrophic lateral sclerosis mouse models

SOD1 loss of function does not influence survival of transgenic SOD1 disease models

The possibility that SOD1 loss of function contributes to ALS pathogenesis has been investigated by analysing the double mutant progeny of *Sod1*$^{-/-}$ or of *Ccs*$^{-/-}$ mice crossed to three tg*SOD1*-ALS lines overexpressing the human mutations, G93A, G37R and G85R. These crosses produce double mutant mice in which either the transgenic human mutant protein is the only SOD1 present (in the case of *Sod1*$^{-/-}$ crosses) or both the endogenous and transgenically expressed SOD1 are mostly inactive due to the *Ccs*$^{-/-}$ background.

As both G93A and G37R mutant SOD1 retain dismutase activity (Fig. 1) they are not informative for the effect of SOD1 dismutase loss of function when on a *Sod1* null background. Further, transgenes often form multiple copy concatamers and indeed both the tg*SOD1*^{G93A} and the tg*SOD1*^{G37R} lines have an increase of $>$6-fold of mouse SOD1 activity, compared with non-transgenic control mice (Bruijn *et al*., 1997; Subramaniam *et al*., 2002; Deng *et al*., 2006). As a result, even on a *Ccs*$^{-/-}$ background, these two transgenic lines still have SOD1 activity levels comparable with those of non-transgenic wild-type mice and so are not useful for examining the effects of SOD1 loss of function on disease course (Subramaniam *et al*., 2002).

However, two experiments do examine the effect of mouse SOD1 loss of function on the disease developed by tg*SOD1*-ALS mouse models. These assess progeny from crosses of *Sod1*$^{-/-}$ and *Ccs*$^{-/-}$ with tg*SOD1*^{G85R}, a human ALS mutation that has no detectable intrinsic activity (Borchelt *et al*., 1994; Bruijn *et al*., 1997). Activity is predicted to fall to 0% when crossed with *Sod1*$^{-/-}$ (Bruijn *et al*., 1998) and shown to be 20% when crossed with *Ccs*$^{-/-}$, as expected, given the residual SOD1 activity of the *Ccs*$^{-/-}$ line (Subramaniam *et al*., 2002).

The tg*SOD1*^{G85R} line shows clinical signs of disease between 8–10 months of age, which aggressively progress to paralysis within a few weeks (Bruijn *et al*., 1997). The disease onset is much later than many other tg*SOD1*-ALS mouse models, this is appropriate for evaluating any potential modifying effect of SOD1 loss of function. Crosses to both *Sod1*$^{-/-}$ ($n = 5$ double mutant progeny) and *Ccs*$^{-/-}$ ($n = 10$ double mutant progeny) did not show significant effects on lifespan (Bruijn *et al*., 1997; Subramaniam *et al*., 2002).

Both of these studies resulted in seminal papers that have been extremely important to the field and have answered critical questions about SOD1 gain of function in ALS. However, neither paper answered the separate question about whether loss of function modifies ALS, presumably and quite reasonably because that was not the focus of the papers. Both studies were performed using a cohort size too small to detect potential subtle changes [$n = 5$ in Bruijn *et al*. (1997) and $n = 10$ in Subramaniam *et al*. (2002)] and the sex of the mice analysed was not reported, although it is clear that in both humans and mouse, gender plays a role in ALS natural history (Acevedo-Arozena *et al*., 2011; Joyce *et al*., 2011). Furthermore, neither study evaluated the age of disease onset or performed behavioural analysis therefore not addressing the possibility that SOD1 loss of function may have an impact on onset and disease course. Lastly, both studies lack a quantitative pathology analysis: Bruijn *et al*. (1997) noted $n = 2$ for axonal count in the L5 ventral roots, and Subramaniam *et al*. (2002) perform a qualitative analysis only, at end-stage, therefore missing any potential modifier effect occurring during the disease process.

Thus while it remains unclear if SOD1 loss of function modifies important disease characteristics such as age of onset or progression, it appears not to affect lifespan. The lack of effect on survival in the absence of mouse SOD1 activity is an important result because it shows that life expectancy in this line is determined uniquely by mutant SOD1. It has been also speculated that the lack of an effect on lifespan is due to the insufficient levels of endogenous SOD1 to counteract the oxidative stress present in the tg*SOD1*^{G85R} mice, and because the tg*SOD1*^{G85R} mice die before developing a significant oxidative stress-mediated motor axonopathy (Wang *et al*., 2012). With respect to onset and disease course, a larger cohort would be required to note differences, particularly if they are subtle.

SOD1 activity may influence disease course of transgenic SOD1 disease models

While the effect of mouse SOD1 activity on disease onset and progression of tg*SOD1*-ALS models is unclear from the crosses to *Sod1* null mice, there are other experimental data indicating that SOD1 activity may play a role in modifying SOD1-familial ALS.

SOD1 overexpression and influence on disease

The effect of overexpression of wild-type human SOD1 on disease course has been tested by crossing mutant tg*SOD1*-ALS animals with transgenic mice overexpressing wild-type human SOD1 (tg*SOD1*-WT). Double mutant progeny carry both the wild-type and mutant human *SOD1* transgenes, with two copies of the endogenous mouse *Sod1* in the genetic background (Bruijn *et al*., 1998; Jaarsma *et al*., 2000; Fukada *et al*., 2001; Deng *et al*., 2006; Wang *et al*., 2009*a*, *b*; Prudencio *et al*., 2010). Generally, a worsening of both age of onset and survival have been reported, compared with single mutant tg*SOD1*-ALS littermates. However, we note that tg*SOD1*-WT mice spontaneously develop motor neuron and axon loss and have misfolded SOD1 accumulations (Jaarsma *et al*., 2000) and develop an ALS-like disease when

expression is further increased (Graffmo *et al*., 2013), making the results of these crosses hard to interpret.

Of note, transgenic mice over-expressing CCS have also been generated and crossed to different tgSOD1 lines, and results have ranged from no effect to a significant worsening of the phenotype. However, CCS over-expression was shown to have biological effects in the absence of SOD1 enzymatic activation and also to have an influence on the reduced state of SOD1, making these results not helpful for dissecting the role of dismutase activity on disease (Proescher *et al*., 2008; Son *et al*., 2009; Graffmo *et al*., 2013).

Tissue specific expression and inactivation of mutant SOD1 points to a modifying role for dismutase activity

To address questions regarding the cell-autonomy of SOD1-familial ALS, investigators have used Cre-*loxP* technology to conditionally eliminate mutant SOD1 expression in different cell lineages or used specific promoters to overexpress mutant SOD1 in selected cell types. Analysis of their results is beyond our scope here, but generally demonstrates a central role for neurons in the determination of age of onset and disease progression in SOD1-familial ALS, and has also pointed to a role for other cell types, such as astrocytes and microglia in influencing the course of the disease (Ilieva *et al*., 2009).

Cre-*loxP* experiments were conducted using two mutant tg*SOD1* lines: conditional tg$SOD1^{G37R}$, which retains intrinsic dismutase activity, and conditional tg$SOD1^{G85R}$, which lacks activity. Neuronal excision experiments in both lines showed a beneficial effect on disease onset and survival. Interestingly, for consideration of the effects of dismutase activity, significant differences were found between the conditional tg$SOD1^{G37R}$ and conditional tg$SOD1^{G85R}$ lines after mutant *SOD1* excision from microglia and from astrocytes.

A more profound amelioration was observed with the tg$SOD1^{G85R}$ line in both experiments, with an effect on early disease with microglia excision and also on disease onset with excision in astrocytes; neither result occurred in the tg$SOD1^{G37R}$ experiments (Ilieva *et al*., 2009; Wang *et al*., 2009*b*, 2011). A possible explanation for these divergent results has been proposed to lie in differences in dismutase activity (Wang *et al*., 2012). If in addition to the toxic gain of function effects, tg$SOD1^{G37R}$ has neuroprotective effects in microglia and astrocytes due to its enzymatic activity, then a knockdown of tg$SOD1^{G37R}$ expression would have less ameliorative effects on the disease course than knockdown of the inactive $SOD1^{G85R}$ (Wang *et al*., 2009*b*, 2011).

In support of a modifying effect of SOD1 dismutase activity are findings obtained with the excision of tg$SOD1^{G37R}$ from Schwann cells. Excision from the tg$SOD1^{G37R}$ made the disease progression in the mice more severe. Lobsiger *et al*. (2009) proposed that $SOD1^{G37R}$ activity in Schwann cell had a neuroprotective effect. Conversely, excision of the inactive $SOD1^{G85R}$ caused a delay in disease onset, an increased survival and an amelioration of pathology (Wang *et al*., 2012). Furthermore, increasing $SOD1^{G93A}$ expression specifically in Schwann cells of the tg$SOD1^{G93A}$ mouse had a beneficial effect on disease (Turner *et al*., 2010).

In conclusion, the experimental data overall point to a protective role of SOD1 dismutase activity, at least in some cell types, on non-cell autonomous degeneration and disease in SOD1–ALS.

Conclusions

SOD1 loss of function models share many commonalities with amyotrophic lateral sclerosis indicating specific cell-type sensitivities

SOD1 loss of function was initially thought to play a role in ALS due to the discovery of disease-causing mutations in the *SOD1* gene and due to the well-established link between oxidative stress and neurodegeneration (Smith *et al*., 1991; Stadtman 1992; Stadtman and Berlett, 1997). Indeed free radical damage has been shown in CSF, serum and urine from patients with ALS (Smith *et al*., 1998; Simpson *et al*., 2004; Mitsumoto *et al*., 2008) and proteins, lipids and DNA were shown to have elevated oxidative damage in ALS post-mortem material (Shaw *et al*., 1995; Fitzmaurice *et al*., 1996; Shibata *et al*., 2001). As SOD1-familial ALS undoubtedly arises primarily from SOD1 toxic gain of function, the loss of dismutase activity may play a modifying role. The most useful tools to study this possibility *in vivo* have been the $Sod1^{-/-}$ mice. These mice are a model of chronic oxidative stress, but do not develop a disease that models human ALS.

Long term studies of $Sod1^{-/-}$ mice show striking features related to ALS. Notably, these mice develop a progressive distal motor axonopathy and ALS has indeed been postulated to start by affecting the distal portions of the neurons including neuromuscular junctions and axons (Murray *et al*., 2010). The most affected motor units in $Sod1^{-/-}$ mice are fast-twitch, which is in accordance with observations in ALS models (Frey *et al*., 2000). Motor neurons in *Sod1* null mice have an increased susceptibility to injury and importantly, stimuli such as trauma, could play a role in initiating ALS (Pupillo *et al*., 2012) where humans, even if carrying disease-causing mutations, are healthy for decades. Further, in $Sod1^{-/-}$ mice, motor neurons are preferentially affected compared to sensory neurons, recapitulating the selectivity observed clinically and pathologically in ALS.

The neuromuscular phenotype in *Sod1* null mice has been demonstrated to be caused by the lack of SOD1 in the mitochondrial intermembrane space and a related decrease in axonal mitochondrial density (Fischer *et al*., 2011). The involvement of mitochondria in ALS and other forms of motor neuron disease such as spinal muscular atrophy, has been shown in animal and cellular models and indirectly in post-mortem material (Baloh *et al*., 2007*b*; De Vos *et al*., 2007; Acsadi *et al*., 2009; Sasaki *et al*., 2009; Wen *et al*., 2010; Faes and Callewaert, 2011). As described, $Sod1^{-/-}$ mice have other phenotypes that underline the importance of this gene in neuronal ageing and in neurodegeneration. Among these are the spontaneous progressive loss of retinal cells and auditory ganglion neurons, the increased susceptibility to APP induced neurodegeneration and the increased

susceptibility to apoptotic cell death following brain trauma and ischaemic injury.

SOD1 activity is greatly reduced in human SOD1-familial amyotrophic lateral sclerosis

SOD1 activity is generally reduced to approximately half of normal in patients with SOD1-familial ALS, as measured in red blood cells, lymphoblastoid cells and fibroblasts (Fig. 1). Indirect evidence raises the possibility that a more severe reduction could occur in susceptible tissues and cell types, owing to reduced mutant *SOD1* messenger RNA half-life in the CNS and due to possible effects of SOD1 protein misfolding and aggregation on activity.

Data from other human diseases involving loss of enzyme activity shows many of these are recessive and heterozygotes are generally unaffected (Mitchell *et al.*, 2011). However, this is clearly not the case in the *Sod1*$^{+/-}$ mouse; these animals have increased neuronal loss and increased susceptibility to injury. Further, the stimuli to which these mice are more susceptible are motor neuron axonal damage and glutamate toxicity, both closely related to ALS pathogenesis. In addition, the blood–brain barrier is more permeable following injury in these mice; indirect evidence of a similar state has also been described in patients with ALS and in mouse models of ALS. Exacerbation of neurodegenerative phenotypes in an Alzheimer's disease mouse model when on a *Sod1*$^{+/-}$ background also indicates a potential predisposition to neurodegeneration of mice with a 50% reduction in SOD1 activity. Lastly, as *Sod1*$^{+/-}$ mice show a spontaneous loss of spiral ganglion cells this confirms that decreased dismutase activity has direct consequences on neuronal survival.

A human SOD1 loss of function phenotype?

Although both *Sod1*$^{-/-}$ and *Sod1*$^{+/-}$ mice develop characteristics that have obvious relevance to ALS, we have found no data from human genetic analyses to suggest that SOD1 loss of function alone causes the human disease. >155 mutations in SOD1 have been described (Fig. 1), but there have been no truncation mutations occurring in the N-terminal part of SOD1 that would generate an effectively null allele (i.e. due to reduced expression from nonsense mediated decay of the messenger RNA or from inactivity of just a short stretch of N-terminal amino acids). We note a frameshift in exon 2 generating a predicted 35 amino acid protein that might be a null, has been reported in a family with ALS, but data regarding segregation are unclear making conclusions uncertain (Hu *et al.*, 2012).

Further, we could find no description of patients with full loss of SOD1 activity, even when SOD1 mutations are found in homozygosity. Of the six homozygous SOD1 mutations (L84F, N86S, D90A, L117V, L126S and G27delGGACCA) described, activities for four (D90A, L117V, L126S and G27delGGACCA) have been measured and vary between 25% and 93% of normal levels (Andersen *et al.*, 1995; Boukaftane *et al.*, 1998; Hayward *et al.*, 1998; Kato *et al.*, 2001; Zinman *et al.*, 2009; Synofzik *et al.*, 2012). Of note, a patient with a *CCS* homozygous mutation has been described with SOD1 activity of ~25% of normal. This patient showed a complex neurodevelopmental phenotype; however, this is attributed to a mutation in *SLC33A1* and not a loss of SOD1 function (Huppke *et al.*, 2012).

SOD1 gain and loss of function could complement each other in amyotrophic lateral sclerosis pathogenesis

A reduction in SOD1 activity is not causative for ALS (which is certainly what the mouse data show), however, it may modify disease, as suggested by results from the mouse cross experiments described above. It seems likely such modifying effects would come through an increased susceptibility to neurodegeneration either directly through, for example, the increased susceptibility to axonal damage seen in *Sod1*$^{+/-}$ mice, or indirectly through, for example, effects on respiration in high energy consumers such as motor neurons—note the recent finding that SOD1 is a critical focus for integrating O_2, glucose and superoxide levels, through casein kinase signalling, to repress respiration and directing energy metabolism, and that this role is independent of its function in oxidative stress (Reddi and Culotta, 2013). Thus SOD1 loss of function will likely have effects on cellular metabolism and, further, as it is still unclear why this enzyme is produced at such high levels, SOD1 may well have other as yet unknown roles in neuronal function.

Loss and gain of function mechanisms coexist in other neurodegenerative diseases as shown in models of Huntington's disease (Zuccato *et al.*, 2010), Parkinson's disease (Winklhofer *et al.*, 2008) and spinocerebellar ataxia 1 (Lim *et al.*, 2008; Crespo-Barreto *et al.*, 2010). Indeed both loss and gain of function have been hypothesized to contribute to pathogenesis in ALS caused by *TARDBP* and *FUS* mutations (Lagier-Tourenne and Cleveland, 2009; Guo *et al.*, 2011).

SOD1 has a crucial role in superoxide clearance and its loss of function generates an increased state of oxidative stress. In a tgSOD1-ALS mouse model, SOD1 is itself a major target of oxidization (Andrus *et al.*, 1998) and SOD1 oxidation and glutathionylation, which occurs in response to oxidative stress, both increase the propensity of the dimer to dissociate and become misfolded (Khare *et al.*, 2004; Rakhit *et al.*, 2004; Ezzi *et al.*, 2007; Wilcox *et al.*, 2009).

These findings set the scene for a potential co-operation of SOD1 loss and gain of function in ALS pathogenesis. Indeed, a vicious circle can be hypothesized in which oxidized SOD1 has an increased propensity to misfold, causing seeding and aggregation of SOD1 and resulting in a reduction of dismutase activity, which therefore feeds more potential oxidative stress to the start of the loop (Fig. 2). We note that the strong link between SOD1 misfolding and its loss of function (see above) make the two effects very difficult to assess independently.

A number of recent findings have underlined how such a mechanism could be relevant not only to SOD1-familial ALS, but also to sporadic cases. Hyperoxidized and misfolded SOD1 have been demonstrated in sporadic ALS cases (Bosco *et al.*, 2010; Forsberg

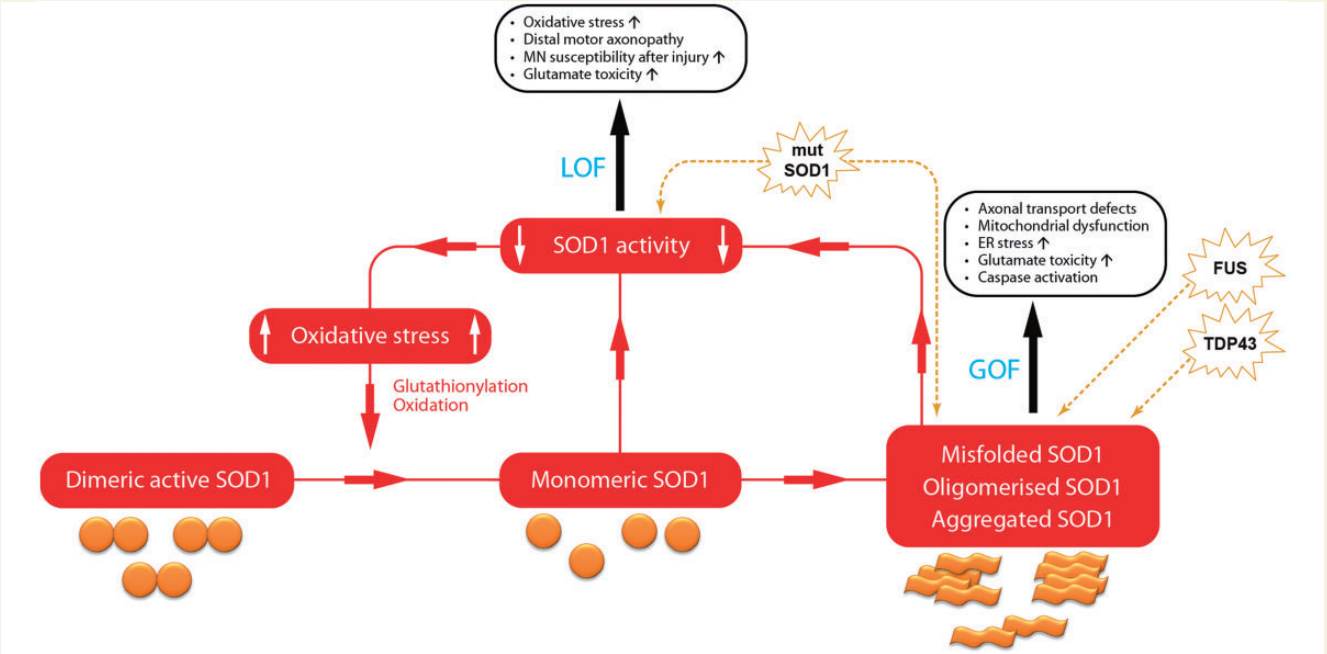

Figure 2 The cycle of SOD1 loss of function, schematic representation of a potential co-operation between SOD1 loss and gain of function in SOD1–familial ALS pathogenesis. SOD1 loss of function (LOF) increases levels of oxidative stress, which through glutathionylation and oxidation, can facilitate the monomerisation of dimeric SOD1. Once monomerized, SOD1 is more prone to become misfolded, oligomerized and aggregated. The monomerization of previously active dimeric SOD1 and the recruitment of SOD1 into aggregates further enhance the loss of function, feeding back to the beginning of the loop. In this way the gain of function (GOF) effects of misfolded, oligomerized and aggregated SOD1, which are known to cause motor neuron degeneration, are amplified by the loss of function circle. Mutant SOD1 (mutSOD1) has both a direct effect on reduction of SOD1 activity and induces SOD1 misfolding and aggregation. Mislocalisation of both TDP43 and FUS result in misfolding of SOD1. ER = endoplasmic reticulum; MN = motor neuron.

et al., 2010; Guareschi *et al*., 2012) and misfolding of SOD1 was shown to be induced by both TDP43 and FUS mislocalization (Pokrishevsky *et al*., 2012), events that occur in the majority of sporadic patients with ALS (Maekawa *et al*., 2009; Deng *et al*., 2010; Matsuoka *et al*., 2011). Recent studies demonstrating that SOD1 aggregation can be seeded *in vitro* from mouse tg*SOD1*^{G93A} spinal cord material (Chia *et al*., 2010), and 'transmitted' between cells (Munch *et al*., 2011) extend the potential role for these pathogenic mechanisms to the clinical and pathological 'spread' of ALS (Ravits *et al*., 2007; Pokrishevsky *et al*., 2012). Of note, SOD1 was also found to be oxidized in Alzheimer's disease and Parkinson's disease (Choi *et al*., 2005).

In fact, it is possible to speculate that the absence of SOD1 in the loss of function mouse model omits one of the most important targets of oxidative stress in ALS—that is, SOD1 itself—leaving the pathogenic cascade incomplete.

Finally, we note the lack of studies addressing the expression of the SOD1 *trans* allele in SOD1–familial ALS and the possibility that this plays a role in modifying the disease. Indeed, a 50 base pair deletion in the promoter region of SOD1 has been described to influence SOD1 expression and there have been attempts to correlate this with clinical characteristics in sporadic ALS, although so far results have not been replicated (Broom *et al*., 2008). To our knowledge no studies analyse the SOD1 trans-allele in SOD1–familial ALS cases for the presence of this variant or other factors influencing the expression of the trans-allele.

Implications of SOD1 loss of function for current therapeutic approaches for amyotrophic lateral sclerosis and other diseases

Therapies are being developed for ALS and other neurodegenerative disease caused by dominant mutations, which entail knockdown of the mutant allele RNA (Smith *et al*., 2006; Kordasiewicz *et al*., 2012; Lu and Yang 2012). This approach is showing promise for Huntington's disease; a recent report has shown suppression of huntingtin in Huntington's disease mouse models and in the non-human primate brain, and a 75% suppression of huntingtin throughout the CNS appears to be well tolerated (Kordasiewicz *et al*., 2012).

A number of analogous strategies have been tested for *SOD1* (Ralph *et al*., 2005; Raoul *et al*., 2005; Saito *et al*., 2005; Smith *et al*., 2006; Wang *et al*., 2010; Towne *et al*., 2011; Wright *et al*., 2012) and have shown very encouraging results. Excitingly, a phase 1 clinical trial has been conducted in *SOD1*-ALS (Fratta, 2013; Miller *et al*., 2013) using antisense oligonucleotides that silence both mutant and wild-type SOD1, that were previously shown to be effective in a transgenic *SOD1*-ALS rat model (Smith *et al*., 2006). The main aim of this study, the first of its kind, was to assess safety and so the treatment was undertaken for periods too brief to obtain a biological effect on SOD1 levels, so although the regime is reported

to be well-tolerated, it remains to be determined whether SOD1 downregulation causes unwanted effects.

The *Sod1* knockout mouse data presented here are important for these types of studies in illustrating the need to understand the full implications of such strategies. These are not only neuronal; for example, the reason most *Sod1* null (and a small percentage of $Sod1^{+/-}$) mice die is liver cancer. Thus particular attention must be paid to delivery routes, protein levels and distribution.

We note that the null mice lack *Sod1* completely, from the earliest time in development, and no appropriate model appears to be available currently for evaluating the effects of long-term endogenous *Sod1* knockdown from adulthood. The preclinical trials with RNA interference technologies used tg*SOD1*-ALS adult models to investigate SOD1 reduction on a very early onset and fast progressing disease, but did not study potential long term effects (Raoul *et al*., 2005; Smith *et al*., 2006). Experiments using conditional *Sod1* alleles would help clarify the situation. A hopeful note for ALS from the Huntington's disease study is that transient knockdown ameliorates disease for an extended period (Kordasiewicz *et al*., 2012).

The data from $Sod1^{+/-}$ animals indicate that in patients with SOD1–ALS there may be a case for epidemiological studies of the known phenotypes that arise with a 50% enzyme loss; for example, is there a greater incidence of cardiovascular disease and stroke in families with SOD1–familial ALS? Do these families have an increased incidence of liver cancer or, protection from tumours such as lung cancer, given that the majority of human lung adenocarcinomas express SOD1 at higher than normal levels (Somwar *et al*., 2011).

Finally, knowledge of the effects of SOD1 loss of activity clearly indicates that this gene should be screened in cohorts with diseases such as hereditary distal motor neuropathies, age-related macular degeneration and progressive hearing loss, because both homozygous and heterozygous loss of function are compatible with life, at least in mice, but have abnormal phenotypes.

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Supplementary material

Supplementary material is available at *Brain* online.

References

Acevedo-Arozena A, Kalmar B, Essa S, Ricketts T, Joyce P, Kent R, et al. A comprehensive assessment of the SOD1G93A low-copy transgenic mouse, which models human amyotrophic lateral sclerosis. Dis Model Mech 2011; 4: 686–700.

Acsadi G, Lee I, Li X, Khaidakov M, Pecinova A, Parker GC, et al. Mitochondrial dysfunction in a neural cell model of spinal muscular atrophy. J Neurosci Res 2009; 87: 2748–56.

Andersen PM, Al Chalabi A. Clinical genetics of amyotrophic lateral sclerosis: what do we really know? Nat Rev Neurol 2011; 7: 603–15.

Andersen PM, Nilsson P, Ala-Hurula V, Keranen ML, Tarvainen I, Haltia T, et al. Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in CuZn-superoxide dismutase. Nat Genet 1995; 10: 61–6.

Andrus PK, Fleck TJ, Gurney ME, Hall ED. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 1998; 71: 2041–8.

Apostolski S, Nikolic J, Bugarski-Prokopljevic C, Miletic V, Pavlovic S, Filipovic S. Serum and CSF immunological findings in ALS. Acta Neurol Scand 1991; 83: 96–8.

Baloh RH, Rakowicz W, Gardner R, Pestronk A. Frequent atrophic groups with mixed-type myofibers is distinctive to motor neuron syndromes. Muscle Nerve 2007a; 36: 107–10.

Baloh RH, Schmidt RE, Pestronk A, Milbrandt J. Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-Tooth disease from mitofusin 2 mutations. J Neurosci 2007b; 27: 422–30.

Barber SC, Shaw PJ. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. Free Radic Biol Med 2010; 48: 629–41.

Beal MF. Role of excitotoxicity in human neurological disease. Curr Opin Neurobiol 1992; 2: 657–62.

Beckman JS, Carson M, Smith CD, Koppenol WH. ALS, SOD and peroxynitrite. Nature 1993; 364: 584.

Bhusari SS, Dobosy JR, Fu V, Almassi N, Oberley T, Jarrard DF. Superoxide dismutase 1 knockdown induces oxidative stress and DNA methylation loss in the prostate. Epigenetics 2010; 5: 402–9.

Birve A, Neuwirth C, Weber M, Marklund SL, Nilsson AC, Jonsson PA, et al. A novel SOD1 splice site mutation associated with familial ALS revealed by SOD activity analysis. Hum Mol Genet 2010; 19: 4201–6.

Bodendorf U, Danner S, Fischer F, Stefani M, Sturchler-Pierrat C, Wiederhold KH, et al. Expression of human beta-secretase in the mouse brain increases the steady-state level of beta-amyloid. J Neurochem 2002; 80: 799–806.

Borchelt DR, Lee MK, Slunt HS, Guarnieri M, Xu ZS, Wong PC, et al. Superoxide dismutase 1 with mutations linked to familial amyotrophic lateral sclerosis possesses significant activity. Proc Natl Acad Sci USA 1994; 91: 8292–6.

Bosco DA, Morfini G, Karabacak NM, Song Y, Gros-Louis F, Pasinelli P, et al. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat Neurosci 2010; 13: 1396–403.

Boukaftane Y, Khoris J, Moulard B, Salachas F, Meininger V, Malafosse A, et al. Identification of six novel SOD1 gene mutations in familial amyotrophic lateral sclerosis. Can J Neurol Sci 1998; 25: 192–6.

Bowling AC, Schulz JB, Brown RH Jr., Beal MF. Superoxide dismutase activity, oxidative damage, and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. J Neurochem 1993; 61: 2322–5.

Broom WJ, Greenway M, Sadri-Vakili G, Russ C, Auwarter KE, Glajch KE, et al. 50bp deletion in the promoter for superoxide dismutase 1 (SOD1) reduces SOD1 expression *in vitro* and may correlate with increased age of onset of sporadic amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2008; 9: 229–37.

Browne SE, Bowling AC, Baik MJ, Gurney M, Brown RH Jr., Beal MF. Metabolic dysfunction in familial, but not sporadic, amyotrophic lateral sclerosis. J Neurochem 1998; 71: 281–7.

Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, et al. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron 1997; 18: 327–38.

Bruijn LI, Houseweart MK, Kato S, Anderson KL, Anderson SD, Ohama E, et al. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. Science 1998; 281: 1851–4.

Casaril M, Corso F, Bassi A, Capra F, Gabrielli GB, Stanzial AM, et al. Decreased activity of scavenger enzymes in human hepatocellular carcinoma, but not in liver metastases. International Journal of Clinical and Laboratory research 1994; 24: 94–97.

Cassereau J, Chevrollier A, Gueguen N, Desquiret V, Verny C, Nicolas G, et al. Mitochondrial dysfunction and pathophysiology of Charcot-Marie-Tooth disease involving GDAP1 mutations. Exp Neurol 2011; 227: 31–41.

Chang LY, Slot JW, Geuze HJ, Crapo JD. Molecular immunocytochemistry of the CuZn superoxide dismutase in rat hepatocytes. J Cell Biol 1988; 107: 2169–79.

Chestnut BA, Chang Q, Price A, Lesuisse C, Wong M, Martin LJ. Epigenetic regulation of motor neuron cell death through DNA methylation. J Neurosci 2011; 31: 16619–36.

Chia R, Tattum MH, Jones S, Collinge J, Fisher EM, Jackson GS. Superoxide dismutase 1 and tgSOD1 mouse spinal cord seed fibrils, suggesting a propagative cell death mechanism in amyotrophic lateral sclerosis. PLoS One 2010; 5: e10627.

Choi J, Rees HD, Weintraub ST, Levey AI, Chin LS, Li L. Oxidative modifications and aggregation of Cu,Zn-superoxide dismutase associated with Alzheimer and Parkinson diseases. J Biol Chem 2005; 280: 11648–55.

Crapo JD, Oury T, Rabouille C, Slot JW, Chang LY. Copper,zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci USA 1992; 89: 10405–9.

Crespo-Barreto J, Fryer JD, Shaw CA, Orr HT, Zoghbi HY. Partial loss of ataxin–1 function contributes to transcriptional dysregulation in spinocerebellar ataxia type 1 pathogenesis. PLoS Genet 2010; 6: e1001021.

Culotta VC, Klomp LW, Strain J, Casareno RL, Krems B, Gitlin JD. The copper chaperone for superoxide dismutase. J Biol Chem 1997; 272: 23469–72.

Culotta VC, Lin SJ, Schmidt P, Klomp LW, Casareno RL, Gitlin J. Intracellular pathways of copper trafficking in yeast and humans. Adv Exp Med Biol 1999; 448: 247–54.

de Lustig ES, Serra JA, Kohan S, Canziani GA, Famulari AL, Dominguez RO. Copper-zinc superoxide dismutase activity in red blood cells and serum in demented patients and in aging. J Neurol Sci 1993; 115: 18–25.

De Vos KJ, Chapman AL, Tennant ME, Manser C, Tudor EL, Lau KF, et al. Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. Hum Mol Genet 2007; 16: 2720–8.

Deng HX, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, Hung WY, et al. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. Science 1993; 261: 1047–51.

Deng HX, Shi Y, Furukawa Y, Zhai H, Fu R, Liu E, et al. Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. Proc Natl Acad Sci USA 2006; 103: 7142–7.

Deng HX, Zhai H, Bigio EH, Yan J, Fecto F, Ajroud K, et al. FUS-immunoreactive inclusions are a common feature in sporadic and non-SOD1 familial amyotrophic lateral sclerosis. Ann Neurol 2010; 67: 739–48.

Dengler R, Konstanzer A, Kuther G, Hesse S, Wolf W, Struppler A. Amyotrophic lateral sclerosis: macro-EMG and twitch forces of single motor units. Muscle Nerve 1990; 13: 545–50.

Didion SP, Kinzenbaw DA, Schrader LI, Faraci FM. Heterozygous CuZn superoxide dismutase deficiency produces a vascular phenotype with aging. Hypertension 2006; 48: 1072–9.

Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson RL, Van Remmen H, et al. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene 2005; 24: 367–380.

Ezzi SA, Urushitani M, Julien JP. Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation. J Neurochem 2007; 102: 170–8.

Faes L, Callewaert G. Mitochondrial dysfunction in familial amyotrophic lateral sclerosis. J Bioenerg Biomembr 2011; 43: 587–92.

Fischer LR, Culver DG, Tennant P, Davis AA, Wang M, Castellano-Sanchez A, et al. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. Exp Neurol 2004; 185: 232–40.

Fischer LR, Glass JD. Oxidative stress induced by loss of Cu,Zn-superoxide dismutase (SOD1) or superoxide-generating herbicides causes axonal degeneration in mouse DRG cultures. Acta Neuropathol 2010; 119: 249–59.

Fischer LR, Igoudjil A, Magrané J, Li Y, Hansen JM, Manfredi G, et al. SOD1 targeted to the mitochondrial intermembrane space prevents motor neuropathy in the Sod1 knockout mouse. Brain 2011; 134: 196–209.

Fischer LR, Li Y, Asress SA, Jones DP, Glass JD. Absence of SOD1 leads to oxidative stress in peripheral nerve and causes a progressive distal motor axonopathy. Exp Neurol 2012; 233: 163–71.

Fitzmaurice PS, Shaw IC, Kleiner HE, Miller RT, Monks TJ, Lau SS, et al. Evidence for DNA damage in amyotrophic lateral sclerosis. Muscle Nerve 1996; 19: 797–8.

Flood DG, Reaume AG, Gruner JA, Hoffman EK, Hirsch JD, Lin YG, et al. Hindlimb motor neurons require Cu/Zn superoxide dismutase for maintenance of neuromuscular junctions. Am J Pathol 1999; 155: 663–72.

Forsberg K, Jonsson PA, Andersen PM, Bergemalm D, Graffmo KS, Hultdin M, et al. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One 2010; 5: e11552.

Fratta P. Antisense makes sense for ALS. Lancet Neurol 2013; 12: 416–417.

Frey D, Schneider C, Xu L, Borg J, Spooren W, Caroni P. Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. J Neurosci 2000; 20: 2534–42.

Fridovich I. Biological effects of the superoxide radical. Arch Biochem Biophys 1986; 247: 1–11.

Fridovich I. Superoxide radical and superoxide dismutases. Annu Rev Biochem 1995; 64: 97–112.

Fukada K, Nagano S, Satoh M, Tohyama C, Nakanishi T, Shimizu A, et al. Stabilization of mutant Cu/Zn superoxide dismutase (SOD1) protein by coexpressed wild SOD1 protein accelerates the disease progression in familial amyotrophic lateral sclerosis mice. Eur J Neurosci 2001; 14: 2032–6.

Ge WW, Leystra-Lantz C, Sanelli TR, McLean J, Wen W, Strong W, et al. Neuronal tissue-specific ribonucleoprotein complex formation on SOD1 mRNA: alterations by ALS SOD1 mutations. Neurobiol Dis 2006; 23: 342–50.

Graffmo KS, Forsberg K, Bergh J, Birve A, Zetterstrom P, Andersen P, et al. Expression of wild-type human superoxide dismutase-1 in mice causes amyotrophic lateral sclerosis. Human Mol Genet 2013; 22: 51–60.

Guareschi S, Cova E, Cereda C, Ceroni M, Donetti E, Bosco DA, et al. An over-oxidized form of superoxide dismutase found in sporadic amyotrophic lateral sclerosis with bulbar onset shares a toxic mechanism with mutant SOD1. Proc Natl Acad Sci USA 2012; 109: 5074–9.

Guo W, Chen Y, Zhou X, Kar A, Ray P, Chen X, et al. An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity. Nat Struct Mol Biol 2011; 18: 822–30.

Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 1994; 264: 1772–5.

Haverkamp LJ, Appel V, Appel SH. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. Brain 1995; 118: 707–19.

Hayward C, Brock DJH, Minns RA, Swingler RJ. Homozygosity for Asn86Ser mutation in the CuZn superoxide dismutase gene produces a severe clinical phenotype in a juvenile onset case of familial amyotrophic lateral sclerosis. J Med Genet 1998; 35: 174.

Ho YS, Gargano M, Cao J, Bronson RT, Heimler I, Hutz RJ. Reduced fertility in female mice lacking copper-zinc superoxide dismutase. J Biol Chem 1998; 273: 7765–9.

Hu J, Chen K, Ni B, Li L, Chen G, Shi S. A novel SOD1 mutation in amyotrophic lateral sclerosis with a distinct clinical phenotype. Amyotroph Lateral Scler 2012; 13: 149–54.

Huang TT, Yasunami M, Carlson EJ, Gillespie AM, Reaume AG, Hoffman EK, et al. Superoxide-mediated cytotoxicity in superoxide dismutase-deficient fetal fibroblasts. Arch Biochem Biophys 1997; 344: 424–32.

Huppke P, Brendel C, Korenke GC, Marquardt I, Donsante A, Yi L, et al. Molecular and biochemical characterization of a unique mutation in CCS, the human copper chaperone to superoxide dismutase. Hum Mutat 2012; 33: 1207–15.

Ilieva H, Polymenidou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J Cell Biol 2009; 187: 761–72.

Jaarsma D, Haasdijk ED, Grashorn JA, Hawkins R, van Duijn W, Verspaget HW, et al. Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. Neurobiol Dis 2000; 7: 623–43.

Jang YC, Lustgarten MS, Liu Y, Muller FL, Bhattacharya A, Liang H, et al. Increased superoxide *in vivo* accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration. FASEB J 2010; 24: 1376–90.

Jonsson PA, Bergemalm D, Andersen PM, Gredal O, Brannstrom T, Marklund SL. Inclusions of amyotrophic lateral sclerosis-linked superoxide dismutase in ventral horns, liver, and kidney. Ann Neurol 2008; 63: 671–5.

Jonsson PA, Ernhill K, Andersen PM, Bergemalm D, Brannstrom T, Gredal O, et al. Minute quantities of misfolded mutant superoxide dismutase-1 cause amyotrophic lateral sclerosis. Brain 2004; 127: 73–88.

Joyce PI, Fratta P, Fisher EM, Acevedo-Arozena A. SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mamm Genome 2011; 22: 420–48.

Kato M, Aoki M, Ohta M, Nagai M, Ishizaki F, Nakamura S, et al. Marked reduction of the Cu/Zn superoxide dismutase polypeptide in a case of familial amyotrophic lateral sclerosis with the homozygous mutation. Neurosci Lett 2001; 312: 165–8.

Keithley EM, Canto C, Zheng QY, Wang X, Fischel-Ghodsian N, Johnson KR. Cu/Zn superoxide dismutase and age-related hearing loss. Hear Res 2005; 209: 76–85.

Keller GA, Warner TG, Steimer KS, Hallewell RA. Cu,Zn superoxide dismutase is a peroxisomal enzyme in human fibroblasts and hepatoma cells. Proc Natl Acad Sci USA 1991; 88: 7381–5.

Kennel PF, Finiels F, Revah F, Mallet J. Neuromuscular function impairment is not caused by motor neurone loss in FALS mice: an electromyographic study. Neuroreport 1996; 7: 1427–31.

Khare SD, Caplow M, Dokholyan NV. The rate and equilibrium constants for a multistep reaction sequence for the aggregation of superoxide dismutase in amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 2004; 101: 15094–9.

Kobayashi J, Kuroda M, Kawata A, Mochizuki Y, Mizutani T, Komori T, et al. Novel G37V mutation of SOD1 gene in autopsied patient with familial amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2012; 13: 570–72.

Kondo T, Reaume AG, Huang TT, Carlson E, Murakami K, Chen SF, et al. Reduction of CuZn-superoxide dismutase activity exacerbates neuronal cell injury and edema formation after transient focal cerebral ischemia. J Neurosci 1997; 17: 4180–9.

Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron 2012; 74: 1031–44.

Kostrominova TY. Advanced age-related denervation and fiber-type grouping in skeletal muscle of SOD1 knockout mice. Free Radic Biol Med 2010; 49: 1582–93.

Lagier-Tourenne C, Cleveland DW. Rethinking ALS: the FUS about TDP-43. Cell 2009; 136: 1001–4.

Larkin LM, Davis CS, Sims-Robinson C, Kostrominova TY, Remmen HV, Richardson A, et al. Skeletal muscle weakness due to deficiency of CuZn-superoxide dismutase is associated with loss of functional innervation. Am J Physiol Regul Integr Comp Physiol 2011; 301: R1400–7.

Leonardi A, Abbruzzese G, Arata L, Cocito L, Vische M. Cerebrospinal fluid (CSF) findings in amyotrophic lateral sclerosis. J Neurol 1984; 231: 75–8.

Liaw KY, Lee PH, Wu FC, Tsai JS, Lin-Shiau SY. Zinc, copper, and superoxide dismutase in hepatocellular carcinoma. Am J Gastroenterol 1997; 92: 2260–2263.

Lim J, Crespo-Barreto J, Jafar-Nejad P, Bowman AB, Richman R, Hill DE, et al. Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature 2008; 452: 713–8.

Lin MT, Wang MY, Liaw KY, Lee PH, Chien SF, Tsai JS, et al. Superoxide dismutase in hepatocellular carcinoma affects patient prognosis. Hepatogastroenterology 2001; 48: 1102–1105.

Lobsiger CS, Boillee S, McAlonis-Downes M, Khan AM, Feltri ML, Yamanaka K, et al. Schwann cells expressing dismutase active mutant SOD1 unexpectedly slow disease progression in ALS mice. Proc Natl Acad Sci USA 2009; 106: 4465–70.

Lu XH, Yang XW. "Huntingtin Holiday": Progress toward an antisense therapy for Huntington's disease. Neuron 2012; 74: 964–6.

Maekawa S, Leigh PN, King A, Jones E, Steele JC, Bodi I, et al. TDP-43 is consistently co-localized with ubiquitinated inclusions in sporadic and Guam amyotrophic lateral sclerosis but not in familial amyotrophic lateral sclerosis with and without SOD1 mutations. Neuropathology 2009; 29: 672–83.

Marikovsky M, Ziv V, Nevo N, Harris-Cerruti C, Mahler O. Cu/Zn superoxide dismutase plays important role in immune response. J Immunol 2003; 170: 2993–3001.

Marklund SL, Holme E, Hellner L. Superoxide dismutase in extracellular fluids. Clin Chim Acta 1982; 126: 41–51.

Matsuoka T, Fujii N, Kondo A, Iwaki A, Hokonohara T, Honda H, et al. An autopsied case of sporadic adult-onset amyotrophic lateral sclerosis with FUS-positive basophilic inclusions. Neuropathology 2011; 31: 71–6.

Matzuk MM, Dionne L, Guo Q, Kumar TR, Lebovitz RM. Ovarian function in superoxide dismutase 1 and 2 knockout mice. Endocrinology 1998; 139: 4008–11.

Miller T, Pestronk A, David W, Rothstein J, Simpson E, Appel SH, et al. A Phase I, randomised, first-in-human study of an antisense oligonucleotide directed against SOD1 delivered intrathecally in SOD1–familial ALS patients. Lancet Neurol 2013; 12: 435–442.

Mitchell JJ, Trakadis YJ, Scriver CR. Phenylalanine hydroxylase deficiency. Genet Med 2011; 13: 697–707.

Mitsumoto H, Santella RM, Liu X, Bogdanov M, Zipprich J, Wu HC, et al. Oxidative stress biomarkers in sporadic ALS. Amyotroph Lateral Scler 2008; 9: 177–83.

Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. J Biol Chem 2004; 279: 49064–73.

Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R, et al. Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. Free Radic Biol Med 2006; 40: 1993–2004.

Munch C, O'Brien J, Bertolotti A. Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. Proc Natl Acad Sci USA 2011; 108: 3548–53.

Murray LM, Talbot K, Gillingwater TH. Review: neuromuscular synaptic vulnerability in motor neurone disease: amyotrophic lateral sclerosis and spinal muscular atrophy. Neuropathol Appl Neurobiol 2010; 36: 133–56.

Pasinelli P, Brown RH. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. Nat Rev Neurosci 2006; 7: 710–23.

Pokrishevsky E, Grad LI, Yousefi M, Wang J, Mackenzie IR, Cashman NR. Aberrant localization of FUS and TDP43 is associated with misfolding of SOD1 in amyotrophic lateral sclerosis. PLoS One 2012; 7: e35050.

Pramatarova A, Figlewicz DA, Krizus A, Han FY, Ceballos-Picot I, Nicole A, et al. Identification of new mutations in the Cu/Zn superoxide dismutase gene of patients with familial amyotrophic lateral sclerosis. Am J Genet 1995; 56: 592–6.

Proescher JB, Son M, Elliott JL, Culotta VC. Biological effects of CCS in the absence of SOD1 enzyme activation: implications for disease in a mouse model for ALS. Hum Mol Genet 2008; 17: 1728–37.

Prudencio M, Durazo A, Whitelegge JP, Borchelt DR. An examination of wild-type SOD1 in modulating the toxicity and aggregation of ALS-associated mutant SOD1. Hum Mol Genet 2010; 19: 4774–89.

Pun S, Santos AF, Saxena S, Xu L, Caroni P. Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. Nat Neurosci 2006; 9: 408–19.

Pupillo E, Messina P, Logroscino G, Zoccolella S, Chio A, Calvo A, et al. Trauma and amyotrophic lateral sclerosis: a case-control study from a population-based registry. Eur J Neurol 2012; 19: 1509–17.

Rakhit R, Crow JP, Lepock JR, Kondejewski LH, Cashman NR, Chakrabartty A. Monomeric Cu,Zn-superoxide dismutase is a common misfolding intermediate in the oxidation models of sporadic and familial amyotrophic lateral sclerosis. J Biol Chem 2004; 279: 15499–504.

Ralph GS, Radcliffe PA, Day DM, Carthy JM, Leroux MA, Lee DC, et al. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. Nat Med 2005; 11: 429–33.

Raoul C, Abbas-Terki T, Bensadoun JC, Guillot S, Haase G, Szulc J, et al. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. Nat Med 2005; 11: 423–8.

Ratovitski T, Corson LB, Strain J, Wong P, Cleveland DW, Culotta VC, et al. Variation in the biochemical/biophysical properties of mutant superoxide dismutase 1 enzymes and the rate of disease progression in familial amyotrophic lateral sclerosis kindreds. Hum Mol Genet 1999; 8: 1451–60.

Ravits J, Laurie P, Fan Y, Moore DH. Implications of ALS focality: rostral-caudal distribution of lower motor neuron loss postmortem. Neurology 2007; 68: 1576–82.

Reaume AG, Elliott JL, Hoffman EK, Kowall NW, Ferrante RJ, Siwek DF, et al. Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. Nat Genet 1996; 13: 43–7.

Reddi AR, Culotta VC. SOD1 integrates signals from oxygen and glucose to repress respiration. Cell 2013; 152: 224–35.

Robberecht W, Sapp P, Viaene MK, Rosen D, McKenna-Yasek D, Haines J, et al. Cu/Zn superoxide dismutase activity in familial and sporadic amyotrophic lateral sclerosis. J Neurochem 1994; 62: 384–7.

Roe JA, Butler A, Scholler DM, Valentine JS, Marky L, Breslauer KJ. Differential scanning calorimetry of Cu,Zn-superoxide dismutase, the apoprotein, and its zinc-substituted derivatives. Biochemistry 1988; 27: 950–8.

Rosen DR, Bowling AC, Patterson D, Usdin TB, Sapp P, Mezey E, et al. A frequent ala 4 to val superoxide dismutase-1 mutation is associated with a rapidly progressive familial amyotrophic lateral sclerosis. Hum Mol Genet 1994; 3: 981–7.

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993; 362: 59–62.

Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Ann Neurol 2009; 65 (Suppl 1): S3–9.

Saito Y, Yokota T, Mitani T, Ito K, Anzai M, Miyagishi M, et al. Transgenic small interfering RNA halts amyotrophic lateral sclerosis in a mouse model. J Biol Chem 2005; 280: 42826–30.

Sasaki S, Aoki M, Nagai M, Kobayashi M, Itoyama Y. Mitochondrial alterations in transgenic mice with an H46R mutant Cu/Zn superoxide dismutase gene. J Neuropathol Exp Neurol 2009; 68: 365–73.

Sato T, Nakanishi T, Yamamoto Y, Andersen PM, Ogawa Y, Fukada K, et al. Rapid disease progression correlates with instability of mutant SOD1 in familial ALS. Neurology 2005; 65: 1954–7.

Schwartz PJ, Reaume A, Scott R, Coyle JT. Effects of over- and under-expression of Cu,Zn-superoxide dismutase on the toxicity of glutamate analogs in transgenic mouse striatum. Brain Res 1998; 789: 32–39.

Shaw PJ, Ince PG, Falkous G, Mantle D. Oxidative damage to protein in sporadic motor neuron disease spinal cord. Ann Neurol 1995; 38: 691–5.

Shefner JM, Reaume AG, Flood DG, Scott RW, Kowall NW, Ferrante RJ, et al. Mice lacking cytosolic copper/zinc superoxide dismutase display a distinctive motor axonopathy. Neurology 1999; 53: 1239–46.

Shibata N, Nagai R, Uchida K, Horiuchi S, Yamada S, Hirano A, et al. Morphological evidence for lipid peroxidation and protein glycoxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. Brain Res 2001; 917: 97–104.

Simpson EP, Henry YK, Henkel JS, Smith RG, Appel SH. Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. Neurology 2004; 62: 1758–65.

Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, et al. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci USA 1991; 88: 10540–3.

Smith RA, Miller TM, Yamanaka K, Monia BP, Condon TP, Hung G, et al. Antisense oligonucleotide therapy for neurodegenerative disease. J Clin Invest 2006; 116: 2290–6.

Smith RG, Henry YK, Mattson MP, Appel SH. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. Ann Neurol 1998; 44: 696–9.

Somwar R, Erdjument-Bromage H, Larsson E, Shum D, Lockwood WW, Yang G, et al. Superoxide dismutase 1 (SOD1) is a target for a small molecule identified in a screen for inhibitors of the growth of lung adenocarcinoma cell lines. Proc Natl Acad Sci USA 2011; 108: 16375–80.

Son M, Fu Q, Puttaparthi K, Matthews CM, Elliott JL. Redox susceptibility of SOD1 mutants is associated with the differential response to CCS over-expression *in vivo*. Neurobiol Dis 2009; 34: 155–62.

Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008; 319: 1668–72.

Stadtman ER. Protein oxidation and aging. Science 1992; 257: 1220–4.

Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. Chem Res Toxicol 1997; 10: 485–94.

Sturtz LA, Diekert K, Jensen LT, Lill R, Culotta VC. A fraction of yeast Cu,Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage. J Biol Chem 2001; 276: 38084–9.

Subramaniam JR, Lyons WE, Liu J, Bartnikas TB, Rothstein J, Price DL, et al. Mutant SOD1 causes motor neuron disease independent of copper chaperone-mediated copper loading. Nat Neurosci 2002; 5: 301–7.

Synofzik M, Ronchi D, Keskin I, Basak AN, Wilhelm C, Gobbi C, et al. Mutant superoxide dismutase-1 indistinguishable from wild-type causes ALS. Hum Mol Genet 2012; 21: 3568–74.

Takahashi M, Dinse GE, Foley JF, Hardisty JF, Maronpot RR. Comparative prevalence, multiplicity, and progression of spontaneous and vinyl carbamate-induced liver lesions in five strains of male mice. Toxicol Pathology 2002; 30: 599–605.

Towne C, Setola V, Schneider BL, Aebischer P. Neuroprotection by gene therapy targeting mutant SOD1 in individual pools of motor neurons does not translate into therapeutic benefit in fALS mice. Mol Ther 2011; 19: 274–83.

Turner BJ, Ackerley S, Davies KE, Talbot K. Dismutase-competent SOD1 mutant accumulation in myelinating Schwann cells is not detrimental to normal or transgenic ALS model mice. Hum Mol Genet 2010; 19: 815–24.

Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. Prog Neurobiol 2008; 85: 94–134.

Wang J, Xu G, Borchelt DR. Mapping superoxide dismutase 1 domains of non-native interaction: roles of intra- and intermolecular disulfide bonding in aggregation. J Neurochem 2006; 96: 1277–88.

Wang L, Deng HX, Grisotti G, Zhai H, Siddique T, Roos RP. Wild-type SOD1 overexpression accelerates disease onset of a G85R SOD1 mouse. Hum Mol Genet 2009a; 18: 1642–51.

Wang L, Grisotti G, Roos RP. Mutant SOD1 knockdown in all cell types ameliorates disease in G85R SOD1 mice with a limited additional effect over knockdown restricted to motor neurons. J Neurochem 2010; 113: 166–74.

Wang L, Gutmann DH, Roos RP. Astrocyte loss of mutant SOD1 delays ALS disease onset and progression in G85R transgenic mice. Hum Mol Genet 2011; 20: 286–93.

Wang L, Pytel P, Feltri ML, Wrabetz L, Roos RP. Selective knockdown of mutant SOD1 in Schwann cells ameliorates disease in G85R mutant SOD1 transgenic mice. Neurobiol Dis 2012; 48: 52–7.

Wang L, Sharma K, Grisotti G, Roos RP. The effect of mutant SOD1 dismutase activity on non-cell autonomous degeneration in familial amyotrophic lateral sclerosis. Neurobiol Dis 2009b; 35: 234–0.

Wang X, Culotta VC, Klee CB. Superoxide dismutase protects calcineurin from inactivation. Nature 1996; 383: 434–7.

Watanabe Y, Kuno N, Kono Y, Nanba E, Ohama E, Nakashima K, et al. Absence of the mutant SOD1 in familial amyotrophic lateral sclerosis (FALS) with two base pair deletion in the SOD1 gene. Acta Neurol Scand 1997; 95: 167–72.

Wei JP, Srinivasan C, Han H, Valentine JS, Gralla EB. Evidence for a novel role of copper-zinc superoxide dismutase in zinc metabolism. J Biol Chem 2001; 276: 44798–803.

Wen HL, Lin YT, Ting CH, Lin-Chao S, Li H, Hsieh-Li HM. Stathmin, a microtubule-destabilizing protein, is dysregulated in spinal muscular atrophy. Hum Mol Genet 2010; 19: 1766–78.

Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 2010; 5: 51–66.

Wilcox KC, Zhou L, Jordon JK, Huang Y, Yu Y, Redler RL, et al. Modifications of superoxide dismutase (SOD1) in human erythrocytes: a possible role in amyotrophic lateral sclerosis. J Biol Chem 2009; 284: 13940–7.

Winklhofer KF, Tatzelt J, Haass C. The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. EMBO J 2008; 27: 336–49.

Wong PC, Waggoner D, Subramaniam JR, Tessarollo L, Bartnikas TB, Culotta VC, et al. Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. Proc Natl Acad Sci USA 2000; 97: 2886–91.

Wright PD, Wightman N, Huang M, Weiss A, Sapp PC, Cuny GD, et al. A high-throughput screen to identify inhibitors of SOD1 transcription. Front Biosci (Elite Ed) 2012; 4: 2801–8.

Yip PK, Malaspina A. Spinal cord trauma and the molecular point of no return. Mol Neurodegener 2012; 7: 6.

Yoon EJ, Park HJ, Kim GY, Cho HM, Choi JH, Park HY, et al. Intracellular amyloid beta interacts with SOD1 and impairs the enzymatic activity of SOD1: implications for the pathogenesis of amyotrophic lateral sclerosis. Exp Mol Med 2009; 41: 611–7.

Yoshida T, Maulik N, Engelman RM, Ho YS, Das DK. Targeted disruption of the mouse Sod I gene makes the hearts vulnerable to ischemic reperfusion injury. Circ Res 2000; 86: 264–9.

Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 2002; 33: 337–49.

Zinman L, Liu HN, Sato C, Wakutani Y, Marvelle AF, Moreno D, et al. A mechanism for low penetrance in an ALS family with a novel SOD1 deletion. Neurology 2009; 72: 1153–9.

Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiol Rev 2010; 90: 905–81.