

# Amyotrophic lateral sclerosis: update and new developments

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**Abstract:** Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease. It is typically characterized by adult-onset degeneration of the upper and lower motor neurons, and is usually fatal within a few years of onset. A subset of ALS patients has an inherited form of the disease, and a few of the known mutant genes identified in familial cases have also been found in sporadic forms of ALS. Precisely how the diverse ALS-linked gene products dictate the course of the disease, resulting in compromised voluntary muscular ability, is not entirely known. This review addresses the major advances that are being made in our understanding of the molecular mechanisms giving rise to the disease, which may eventually translate into new treatment options.

**Keywords:** amyotrophic lateral sclerosis, neurodegeneration, motor neuron disease, genetics, aging

## Introduction

Amyotrophic lateral sclerosis (ALS), also known as Charcot's disease or Lou Gehrig's disease is the most widespread type of motor neuron disease. Striking later in life, the disease causes degeneration of motor neurons and consequently progressive atrophy of associated muscle tissues and supporting cells. Unlike similar motor neuron diseases that primarily affect only a single subgroup of neurons (eg, Primary Muscular Atrophy or Primary Lateral Sclerosis), ALS patients typically have both lower motor neuron (LMN) and upper motor neuron (UMN) involvement. The symptoms of ALS commonly are muscle weakness and wasting, especially in the limbs, cramps, twitching, and difficulties in speaking. The lifetime risk of acquiring ALS by age 70 is between 1 in 400 and 1 in 1000,<sup>1</sup> and in general, ALS individuals succumb to the disease within 2–3 years due to respiratory failure.

A growing number of ALS-causing genes have been identified recently and are now under investigation, providing promise for increased understanding of the etiology of the disease. *SOD1*, encoding the highly conserved, cytosolic antioxidant enzyme Cu,Zn-superoxide dismutase (Cu,ZnSOD), was the first such gene to be identified with ALS.<sup>2,3</sup> *SOD1* mutations are common in both familial ALS (FALS) and sporadic ALS (SALS), and have been studied in the most depth. Other genes such as *OPTN*<sup>4</sup> or *TARDBP*, *FUS*, and *ANG* (involved in RNA metabolism)<sup>5</sup> were later identified as causative factors in both FALS and SALS. Suggestive of proteolytic dysfunction, *UBQLN2* was recently implicated in ALS,<sup>6,7</sup> and very recently, nucleotide repeat expansions in *C9ORF72*,<sup>8–10</sup> were found to comprise the largest fraction of ALS-causing mutations

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known to date. The present era is an exciting time for ALS research with the major challenge of understanding how these distinct, underlying triggers lead to a common aberrant cellular dyshomeostasis phenotype, resulting in toxic protein aggregates, neuronal death, and subsequently muscle atrophy that ultimately paralyzes the ALS patient.

Only one drug, riluzole, has been approved to treat ALS, which typically provides a meager gain of a few months of survival.<sup>11</sup> With advances in diagnostics and personalized medicine, however, future ALS patients will hopefully find improved treatment regimes to follow for their specific ALS manifestations. In this review, we will focus on the recent breakthroughs that will likely provide new avenues to reach this outcome. These include increased understanding of the basic biology of ALS and progress toward upcoming therapeutics in development.

## Diagnosis of ALS

### Epidemiology

Worldwide, the incidence rate of ALS varies from approximately 0.3–2.5 cases per year per 100,000 persons.<sup>12</sup> Five percent or greater of all cases run in families (FALS),<sup>13</sup> with a range from 2%–15% in different populations,<sup>14</sup> although regional and/or ethnic variations in incidence<sup>15,16</sup> and penetrance<sup>17</sup> complicate the estimation,<sup>18</sup> as do the organization of the studies themselves, being either population- or clinic-based.<sup>19</sup> Aside from family history, the clinical presentation of FALS and SALS can be very similar.<sup>20</sup> The onset for FALS is typically several years before that of SALS, although an exact age is difficult to estimate. In one study, for example, the mean FALS age was 48, as compared to 66 for a population-based group,<sup>21</sup> whereas in another larger study the discrepancy, although still present, was not as large (52 versus 56, respectively).<sup>22</sup> Typically, in SALS cases, but not always in FALS,<sup>21,22</sup> males appear to predominate,<sup>23</sup> but this may vary among ethnic backgrounds and may be trending toward equality with time.<sup>24</sup> The higher incidence of ALS among war veterans and smokers,<sup>25–27</sup> potentially accounts for the increased male risk, in addition to factors such as male hormones.<sup>28</sup> Interestingly, a recent study suggested that a lower-than-average ratio of the index to ring finger is represented in ALS patients.<sup>29</sup> This measurement (termed the 2D:4D ratio) is thought to reflect androgen exposure in the womb<sup>30,31</sup> and therefore postulates a role for prenatal developmental factors in the disease. Sports (soccer and football) and sport-specific effects (soccer, but not basketball or cycling)<sup>32</sup> have also been implicated in ALS disease development.<sup>25,33</sup> Finally, higher body mass index

(up to 30–35) was found to correlate to disease survival,<sup>34</sup> possibly due to the common weight loss phenotype from muscle wasting associated with disease progression. An improved awareness of risk factors and trends for ALS might eventually establish better preventative measures or treatments, especially for those with a family history of the disease.

### Symptom presentation and examination

No single test for diagnosing ALS exists; most cases are established based on symptom presentation, progression, and tests to eliminate overlapping conditions.<sup>35</sup> ALS is typically characterized by combined symptoms of the UMNs and LMNs. The UMNs of the central nervous systems originate in the motor cortex or brainstem and relay motor information to the LMNs. The LMNs are located in the brainstem and spinal cord and relay impulses from the UMNs to the muscles at neuromuscular synapses to innervate skeletal muscles controlling the arms and legs. UMN symptoms include weakness, speech problems, overactive reflexes, spasticity, and inappropriate emotionality; LMN symptoms also include weakness, as well as decreased reflexes, cramps, twitching and muscle wasting.<sup>36,37</sup> Disease onset usually begins in the limbs (termed spinal onset), although about a quarter of ALS patients have “bulbar” onset,<sup>38</sup> the term describing the facial, mouth/jaw, and tongue muscles controlled by the “bulb,” an early name for the lower brainstem. Associated with poorer prognosis, bulbar onset is more common in elderly patients and women.<sup>39,40</sup> A hallmark of ALS is rapid progression, and over time most patients will display both spinal and bulbar features (including emotionality, yawning, jaw jerking, tongue twitching, wasting, drooling, and difficulties swallowing). The El Escorial Criteria are a set of guidelines for ALS diagnosis, frequently used to gauge clinical trial participation and clinical practice. In some cases, though, these criteria may be overly stringent when used in diagnosis.<sup>41</sup>

Diagnosis may be seen as a process of elimination, although family history can also be useful. The battery of tests performed, ie, blood tests, electromyography, magnetic resonance imaging, and nerve conduction studies, can aid in ruling out other conditions.<sup>42</sup> For example, in some patients, creatine kinase activity may be slightly elevated.<sup>43</sup> Cerebrospinal fluid (CSF) examination, on the other hand, is typically normal but can aid in diagnosing conditions such as multiple sclerosis. Furthermore, muscle biopsy can rule out inclusion body myositis.<sup>44</sup> Indeed, a central challenge in ALS diagnosis is distinguishing the many mimics.

These include injuries (eg, herniated disk, spinal compression, or heavy metal poisoning), cervical spondylosis, metabolic problems such as enzyme/vitamin deficiency (B-12 etc), copper deficiency or thyroid problems, stroke, myopathies or neuropathies, inclusion body myositis, infections such as Lyme or HIV, or diseases such as myasthenia gravis, syringomyelia, cancer, Kennedy's disease, Tay-Sachs diseases, or multiple sclerosis, among others.<sup>12,20,36,37,44–46</sup> Misdiagnoses are in fact very common,<sup>20,47</sup> about 10% of patients with other disorders are diagnosed erroneously with ALS.<sup>48,49</sup> These findings may result in incorrect (potentially harmful) treatments, and delays in obtaining the necessary therapies and support and in seizing clinical trial opportunities.

Attempts to identify ALS-specific biomarkers may prove useful. For example, a study examining blood plasma found statistically significant distinctions in a panel of several hundred metabolites among ALS patients, allowing the authors to cleanly separate control patients from diseased patients (on taking or not taking riluzole), and even to sub-classify LMN-affected patients.<sup>50</sup> Such efforts may eventually aid the clinician in more specifically diagnosing motor neuron disease.

## Pathophysiology

### Protein inclusions and cellular dyshomeostasis

Typical hallmarks of ALS revealed from post-mortem examinations of patient brain and spinal cord sections are neuronal atrophy and the presence of cellular inclusions. Inclusions typical of affected cells include the small, cystatin-C and transferrin-immunoreactive Bunina bodies.<sup>51</sup> Also very common are ubiquitinated cellular inclusions, most often skein-like or of the round Lewy-body hyaline variety.<sup>52</sup> The presence of ubiquitin-reactive inclusions is consistent with a very recent study demonstrating that defects in the ubiquitin proteasome system may be a more generalized feature of ALS.<sup>6</sup> Degenerative cellular abnormalities can afflict the motor cortex, the brainstem, the anterior horn of the spinal cord, the lateral and/or anterior corticospinal tracts. Distinct cellular inclusions, suggested by differential protein composition, are observed in ALS arising from different genetic backgrounds (discussed below).

Another common facet of ALS pathophysiology is irregular glutamate metabolism, targeted by riluzole, the only drug approved to treat ALS.<sup>53</sup> Elevated synaptic glutamate can lead to excessive stimulation of glutamate receptors (eg, AMPA and NMDA) on the postsynaptic neuron, resulting in nerve damage and death through excitotoxicity.

Interestingly, the above-described features may also occur in the supporting glia, including astrocytes in which inclusions and downregulation of GLT-1 (also known as EAAT2) glutamate transporter were observed.<sup>54</sup> Other relevant cellular abnormalities in ALS include an increase of p53-mediated apoptosis, impaired axonal transport, and cytoskeletal and mitochondrial dysfunction.<sup>55–58</sup> Additionally, as disease symptoms appear at mid-to-late life, cumulative damage occurring through increased levels of oxidative stress may be a significant contributor to the disease.<sup>59</sup> A recent study analyzing the CSF of ALS patients suggested distinct metabolic signatures discernible between SALS patients and those with *SOD1* and non-*SOD1* FALS. The metabolomes of *SOD1* FALS patients were observed to be more homogeneous than those of non-*SOD1* FALS patients, which were more homogeneous than those of SALS patients.<sup>60</sup> These observations suggest that genetic contributions to the disease may influence ALS physiology.

## FALS and SALS genes

Despite the identification of some ALS-causing genetic defects in individual families, ALS is not a single-pathway, single-gene condition. Therefore in recent years, high throughput, genome wide association studies have become a favored tactic for filling in the significant remaining space of unknown FALS-causing genes.<sup>61</sup> Nonetheless, consistency in reproducing candidate genes had been a problem<sup>62</sup> until the recent, notable exception of the *C9ORF72* gene in the 9p21 locus,<sup>8,9,63</sup> a major ALS breakthrough. The disease subtypes associated with FALS mutations have been assigned designations of ALS1-ALS15 (Table 1). However, several known FALS mutations have now been documented in SALS cases, suggesting a broader role for these gene products in ALS pathogenesis. Although a variety of genes have been implicated in ALS (Table 1), we will focus on this subset of genes, in which genetic lesions can cause and contribute to both FALS and SALS.

### *SOD1*

The *SOD1* gene encodes the cytosolic enzyme Cu,ZnSOD, which is conserved from bacteria to humans. Cu,ZnSOD catalyzes the dismutation of the superoxide ( $O_2^{\cdot-}$ ) radical anion, a toxic byproduct of cellular respiration, to produce molecular oxygen and hydrogen peroxide,<sup>64</sup> with the toxicity of the latter being removed by conversion through a peroxidase or catalase. Over 150 *SOD1* mutations (Figure 1) account for a significant fraction of FALS, and are typically present in about 20% of such cases (ranging from 2.5%–23.5%), as

**Table 1** Common genes involved in ALS

Gene	Locus	Protein	Found in cellular inclusions	ALS subtype	Other
<b>Autosomal dominant FALS genes also implicated in SALS</b>					
<i>SOD1</i>	21q22.1	Cu,Zn superoxide dismutase (SOD)	+	ALS1	Can be recessive in FALS
<i>FUS</i>	16p11.2	Fused in sarcoma (FUS)	+	ALS6	Can be recessive in FALS
<i>ANG</i>	14q11.1	Angiogenin (ANG)		ALS9	Autosomal Dominant or Haploinsufficient
<i>TARDBP</i>	1p36.22	TAR DNA Binding Protein-43 (TDP-43)	+	ALS10	
<i>OPTN</i>	10p13	Optineurin	+	ALS12	Can be recessive in FALS
<i>C9ORF72</i>	9p21	C9ORF72	?	'ALS-FTD'	Newly characterized
<b>Autosomal dominant FALS genes</b>					
<i>ALS3</i>	18q21	ALS3		ALS3	
<i>SETX</i>	9q34.13	Senataxin		ALS4	Can cause juvenile onset
<i>ALS7</i>	20p13	ALS7		ALS7	
<i>VAPB</i>	20q13.33	VAMP-associated protein B	+	ALS8	Can cause juvenile onset
<i>FIG4</i>	6q21	Phosphoinositide 5-phosphatase		ALS11	
<i>VCP</i>	9p13.3	Valosin-containing protein		ALS14	
<b>Autosomal recessive FALS genes</b>					
<i>ALS2</i>	2q33.1	Alsin		ALS2	Can cause juvenile onset
<i>SPG11</i>	15q15.1	Spatacsin		ALS5	Can cause juvenile onset
<b>X-linked dominant FALS gene</b>					
<i>UBQLN2</i>	Xp11.2	Ubiquilin-2	+	ALS15	Can cause juvenile onset
<b>Other genes</b>					
<i>ATXN2</i>	12q24.1	Ataxin-2		ALS13	Increases ALS susceptibility

**Note:** Gene products discussed in the main text, as well as additional FALS and susceptibility genes and relevant characteristics are noted.

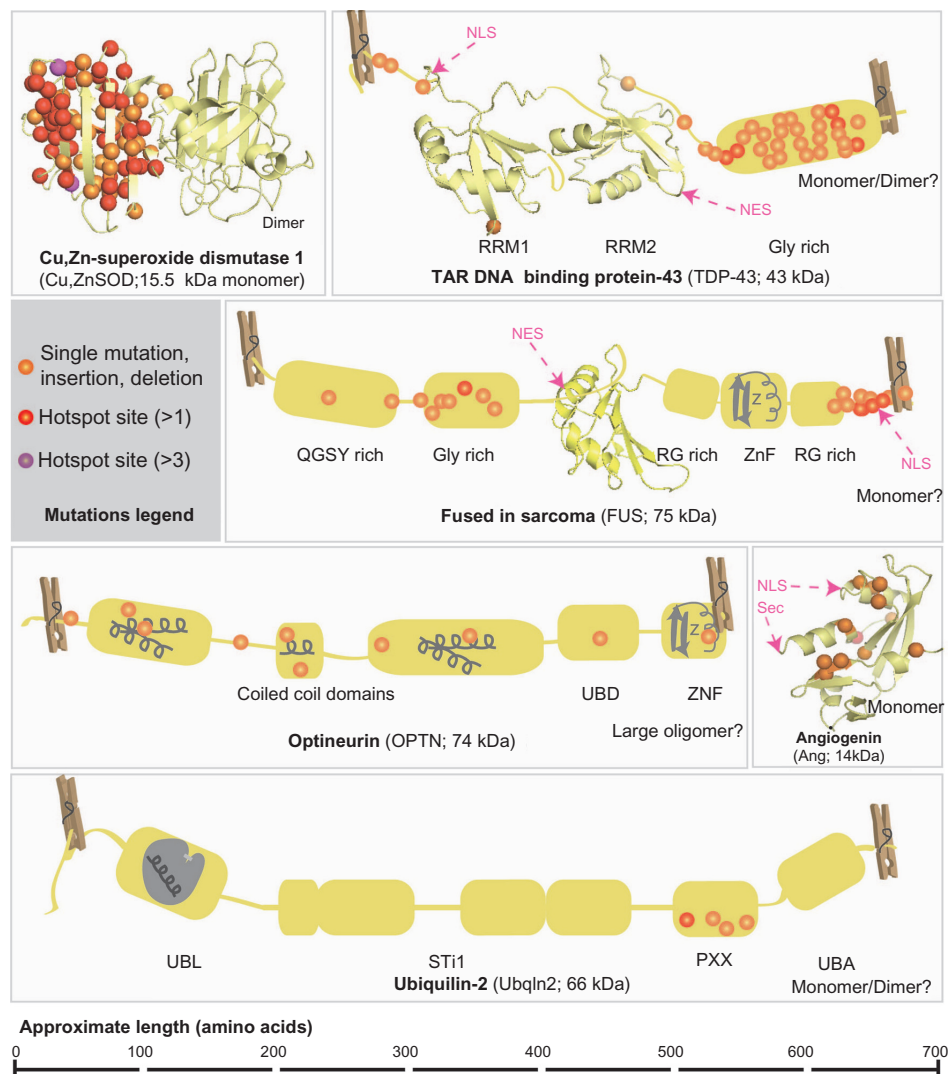
**Abbreviations:** FALS, familial amyotrophic lateral sclerosis; SALS, sporadic amyotrophic lateral sclerosis.

well as in 0.44% to 7% of SALS cases.<sup>19,65</sup> The majority of inherited *SOD1* mutations are dominant, and individuals with two copies of a mutation may have much earlier onset.<sup>66,67</sup> The common D90A *SOD1* mutation is an exception that can be inherited in either a dominant or recessive fashion, as well as appearing sporadically.<sup>68,69</sup>

*SOD1* mutations do not appear to cause disease by a loss of function. For example, transgenic expression of *SOD1* mutants in mice is pathogenic without altering enzyme activity.<sup>70</sup> This is also evidenced by the fact that Cu,ZnSOD deficient mice do not develop motor neuron disease<sup>71</sup> and that mutations are not restricted to the active site of the enzyme.<sup>2</sup> Instead, mutant Cu,ZnSODs form toxic, misfolded species within neuronal and glial Lewy-body like inclusions<sup>72,73</sup> that usually appear before symptom presentation.<sup>73,74</sup> Within these aggregates, mutant Cu,ZnSOD can be associated with heat shock protein Hsc70<sup>75–77</sup> or 14-3-3 proteins, suggesting in the latter case that sequestration of anti-apoptotic proteins could contribute to cell death.<sup>78</sup> In a recent report, strong mutant Cu,ZnSOD immuno-reactivity was observed in small, granular non-ubiquitin reactive inclusions that localize to the cytosol and/or lysosomes of FALS (*SOD1* and non-*SOD1*) and non-*SOD1* SALS patients.<sup>79</sup> Also, Cu,ZnSOD-positive

nuclear inclusions have been observed in spinal-cord derived glia from FALS and SALS patients.<sup>80</sup> Therefore, Cu,ZnSOD aggregates, found in tissues from distinct ALS patients, may be a component of diverse cellular inclusions in affected motor neurons and their supporting cells.

Detailed analyses of Cu,ZnSOD structures and enzymatic mechanisms<sup>81,82</sup> including comparisons to bacterial Cu,ZnSOD<sup>83</sup> and the human mitochondrial MnSOD<sup>84–86</sup> provided an informed foundation to evaluate the diverse mutations.<sup>2,87</sup> To explain the complex effects of Cu,ZnSOD mutations in ALS pathogenesis, we and others have proposed a framework destabilization hypothesis.<sup>87–89</sup> In this hypothesis each of the diverse set of mutations can cause local unfolding events that contribute to a globally defective, self-aggregating protein, which can deleteriously co-aggregate with other cellular proteins.<sup>88</sup> Such framework-destabilizing mutations are associated with other neurodegenerative and cancer prone diseases as typified by mutants of the XPD helicase.<sup>90</sup> Several studies have attempted to characterize the aggregation propensity of mutant forms of Cu,ZnSOD in vitro and in cultured cells, but a direct correlation between mutant protein stability and clinical phenotype has been elusive.<sup>91–94</sup> This lack of correlation could be due to a multitude of contributing



**Figure 1** Known mutations in FALS and SALS-associated proteins.

**Notes:** Known mutations are mapped onto their corresponding proteins. Single mutations can include point mutations, premature stop codons, deletions, or insertions. For simplicity, one of the SOD dimers contains the mapped mutations. Structural and Domain Organization is indicated. Solved structures of domains or entire proteins are shown as ribbon diagrams: Cu,ZnSOD (1PU0); TDP-43 RRM1 (1CQG); TDP-43 RRM2 (1WFO); FUS RRM (1LA6); Angiogenin (1BI1). Clothespins indicate that the tertiary structure and inter-domain associations are not entirely known, so protein is stretched out to better show mutations sites. Schematic depictions of conserved domains without solved structures are shown in grey. Where applicable, known or putative oligomeric state and molecular weights are indicated.

**Abbreviations:** FALS, familial amyotrophic lateral sclerosis; SALS, sporadic amyotrophic lateral sclerosis; NLS, nuclear localization sequence; NES, nuclear export sequence; Sec, cleaved signal sequence; RRM, RNA recognition motif; X rich, X (amino acid residue) rich motifs; UBD, ubiquitin binding domain; ZnF, zinc finger; UBL, ubiquitin like domain; STI1, heat-shock-chaperonin-binding motifs; UBA, ubiquitin associated domain.

factors, ranging from important roles for metals in architectural stability,<sup>95</sup> to aberrant oxidative modifications of the free cysteines,<sup>96,97</sup> to anomalous interactions of mutant Cu,ZnSOD with other cellular components. These components likely include proteins involved in stress responses (eg, Derlin-1, Rac-1)<sup>98,99</sup> folding/maturation (eg, Hsc70 and the Cu,ZnSOD copper chaperone)<sup>77,100</sup> and vesicular transport associated proteins (eg, chromogranin, dynein heavy chain).<sup>101–103</sup>

### TARDBP

The *TARDBP* gene encodes TAR DNA binding protein 43 (TDP-43), a modular DNA/RNA binding protein (Figure 1),

localized to the cytosol and the nucleus, which is involved in splicing and transcriptional regulation.<sup>104</sup> In vivo, TDP-43 depletion in mice resulted in mRNA reduction and splicing errors in many mRNA transcripts and a few non-coding RNAs, particularly long intron-containing transcripts. This suggests a broad role for TDP-43 in alternative splicing and prevention of nonsense-mediated decay of transcripts expressed in neurons.<sup>105</sup> The nearly 40 mutations identified in the *TARDBP* gene encoding TDP-43 (Figure 1) may contribute to up to 6.5% of dominantly-inherited FALS cases,<sup>106,107</sup> in addition to 0%–5% of sporadic cases.<sup>107–110</sup> A reduced nuclear pool of TDP-43 is associated with some mutations,



and cytoplasmic, ubiquitin-reactive hyperphosphorylated TDP-43 inclusions are observed in tissues from frontotemporal dementia (FTD) patients<sup>111,112</sup> and in neuronal and glial tissues samples from SALS and Guam ALS patients.<sup>113</sup> The inclusions commonly co-localize with ubiquitin and the protein p62.<sup>113</sup> However, TDP-43 inclusions are not present in *SOD1* FALS individuals<sup>114</sup> (with the exception of one case<sup>113</sup>) or *FUS* mutant patients.<sup>115</sup>

### FUS

*FUS* encodes fused in sarcoma (FUS, also known as Translated in Liposarcoma, TLS), a modular nucleic acid-associated protein with many similarities to TDP-43, including conservation of protein domains (Figure 1), a role in RNA processing<sup>115</sup> and localization in both the cytosol and nucleus in many cells. About 30 known *FUS* mutations account for approximately 3%–5% of FALS and ~1% of SALS cases<sup>116,117</sup> and all but the one known recessive variant, H517Q<sup>118</sup> cause a dominant phenotype. As with some *TARDBP* mutations, certain *FUS* mutations located near the nuclear localization sequence may shift the nuclear/cytoplasmic balance towards cytosolic. This imbalance occurs by impairing the transportin-mediated import of FUS into the nucleus.<sup>119</sup> FUS-reactive inclusions have been found in tissues from *FUS* mutant FALS patients but not in *SOD1* mutant patients.<sup>115,117</sup> Furthermore, although earlier studies failed to see FUS-immunoreactivity in SALS cases<sup>115</sup> a more recent study did report FUS staining in inclusions from SALS patients.<sup>117</sup> FUS inclusions are commonly seen in FTD patients,<sup>115,118,120</sup> in addition to ALS patients, and these FUS-proteinopathy phenotypes might be distinguished through co-localization of other FUS family member proteins in FTD, but not in ALS.<sup>121</sup> Furthermore, FUS and TDP-43 inclusion phenotypes are thought to be mutually exclusive in FTD,<sup>122,123</sup> but this may not be the case in ALS; although TDP-43 reactivity was not observed in *FUS* ALS mutant tissues,<sup>115</sup> FUS-reactivity was later reported in TDP-43 ALS mutant tissues.<sup>117</sup>

### OPTN

A recent Italian study indicated that approximately 3.5% of SALS patients, in addition to 1.2% of FALS patients, had mutations in the *OPTN* gene,<sup>4</sup> which encodes Optineurin. About a dozen mutations in *OPTN* can lead to ALS, with gain of function mutations dominant and loss of function mutations recessive.<sup>124,125</sup> Optineurin is a multifunctional cytosolic and Golgi-associated coiled-coil domain-containing, ubiquitin-binding phosphoprotein (Figure 1). It is involved in vesicular trafficking and Golgi maintenance, signaling

in the tumor-necrosis factor  $\alpha$ /NF- $\kappa$ B pathway,<sup>126</sup> mGluR signaling<sup>127,128</sup> and autophagy.<sup>129</sup> Optineurin has been shown to form homo-complexes and heteromultimerize with Rab8, myosin VI, and transferrin receptor proteins. In both FALS- and SALS-affected cells, Optineurin can co-localize in inclusion bodies with FUS<sup>130</sup> and TDP-43,<sup>124</sup> although the frequency of such inclusions was shown to be low in another study.<sup>131</sup> Furthermore, Optineurin localization has been observed in basophilic inclusions from *SOD1* FALS patient tissues,<sup>124</sup> although conflictingly this co-localization was not observed in another study in patient-derived or mouse model tissues.<sup>132</sup>

### ANG

Angiogenin (Ang, encoded by the *ANG* gene), a small, hypoxia- and ischemia-inducible<sup>133</sup> ribonuclease A (Figure 1) involved in angiogenesis, is mutated in a smaller number of FALS and SALS cases.<sup>134</sup> Expressed in many tissues, including motor neurons,<sup>135</sup> where it promotes cell survival,<sup>136</sup> Ang is required for the VEGF-mediated stimulation of angiogenesis.<sup>137</sup> Ang is secreted and taken up by effector cells via endocytosis, then translocated to the nucleus, to stimulate transcription of rRNA, among other roles.<sup>135</sup> Due to loss of ribonuclease and/or nuclear translocation activity,<sup>135</sup> *ANG* mutations appear to attenuate angiogenesis although the protein stability is not compromised.<sup>138</sup> Eighteen *ANG* mutations, therefore, can cause a loss-of-function phenotype, with most *ANG* ALS patients presenting with bulbar onset (discussed above).<sup>134</sup>

### UBQLN2

*UBQLN2*, a gene on the X-chromosome, was recently found to be causative for X-linked dominant FALS.<sup>6,139</sup> In affected families, incomplete penetrance was noted in females, presumably due to X-inactivation. The encoded ubiquilin-2 protein (Figure 1) normally performs effector functions in the ubiquitin proteasome pathway by tethering degradation-targeted proteins (through its C-terminal ubiquitin-associated domain) to the proteasome (through association with its N-terminal ubiquitin-like domain). The intervening regions within the protein are less well characterized, and include a PXX (proline-rich) domain, where five distinct mutations were found. In tissues derived from *UBQLN2*-mutant patients, ubiquitin-positive skein-like inclusions were also reactive for ubiquilin 2. This phenotype was particularly notable in the spinal cord and hippocampus, correlating with the appearance of dementia in 20% of the X-linked ALS patients. Furthermore, these inclusions were also positive

for TDP-43, FUS and OPTN, but not Cu,ZnSOD. Notably, ubiquilin-2 inclusion staining was present in all samples from a wide panel of genetically-distinct ALS patient tissues (sporadic, *SOD1*-mutant, *TARDBP* mutant, and non-*FUS*/non-*TARDBP*/non-*SOD1* FALS, and ALS with dementia) but not in non-ALS controls.<sup>6</sup> Expression of mutant ubiquilin-2 protein significantly slowed down proteosomal degradation of a reporter substrate in Neuro-2a cells,<sup>6</sup> suggesting a mechanistic contribution for these mutants. Unlike the other mutations described, those in the *UBQLN2* gene have not yet been implicated in SALS. However, these findings suggest ubiquilin-2 could be generally relevant to ALS pathogenesis.

### C9ORF72

Very recently, two independent research groups flagged *C9ORF72* as the gene at locus 9p21 that was linked to dominant cases of ALS/FTD<sup>8,9</sup> in previous genome-wide association studies. Strikingly, a substantial hexanucleotide repeat (GGGGCC) within an intron of this gene was identified in 24%–46% of FALS cases and 4%–21% of SALS cases, making this the most commonly mutated ALS gene. The expansion appeared to result in nuclear foci and directed preferential splicing of an alternatively spliced transcript.<sup>8</sup> However, precisely how the aberrant RNA metabolism of *C9ORF72* causes ALS is not yet known, and the protein, aside from nuclear localization,<sup>9</sup> has no ascribed function. Interestingly, post-mortem examination of several patients with the *C9ORF72* hexanucleotide repeat, who exhibited ALS and FTD-like symptoms, also revealed neuronal TDP-43 inclusions.<sup>8</sup>

### Commonalities and crosstalk

One puzzle for understanding ALS is that the known ALS-causing gene products have diverse physiological functions. However, some common themes in pathogenesis are beginning to emerge. For example, RNA processing defects are visible in mutants of *TARDBP*, *FUS*, and *ANG* (as well as a FALS gene called *SETX*).<sup>5</sup> Nucleotide repeat expansions have also now been identified in *C9ORF72* (and an ALS-susceptibility protein called Ataxin-2).<sup>140</sup> Proteinacious cellular inclusions are also a common denominator in ALS patient-derived tissues; these can involve ubiquilin-2, as well as SOD, FUS, TDP-43, and/or optineurin. Interestingly however, different disease subtypes appear to reveal aggregates with distinct protein composition. Due to their roles in both ALS and FTD, TDP-43, FUS, OPTN, and ubiquilin-2 have been proposed to function in the context of a unified pathway.<sup>141</sup> Thus, interactions among these components should

be a focus for future research. Along these lines, a recent study in zebrafish found that the expression of human *FUS* could rescue the motor neuron phenotype associated with knockdown of *TARDBP* expression, whereas, conversely, *TARDBP* could not rescue *FUS* knockdown, suggesting that *TARDBP* is genetically upstream of *FUS*.<sup>142</sup> These results are consistent with a study showing that TDP-43 regulates the mRNA processing of *FUS* transcripts as well as its own.<sup>105</sup>

### Genetic overlap between ALS and other diseases

Gene products whose mutations cause ALS have been implicated in other diseases. For example, FUS, TDP-43, ubiquilin-2, and/or optineurin-positive inclusions are found in many FTD patients,<sup>131,143</sup> and *C9ORF72* is implicated also in ALS/FTD.<sup>8,9</sup> TDP-43-immunoreactivity is sometimes seen in hippocampal sclerosis, Pick's disease, and Alzheimer's disease (AD), and ubiquitin staining can occur in the latter disease.<sup>109</sup> Likewise, optineurin has recently been implicated in AD due to its inclusion body staining in neurofibrillary tangles.<sup>144</sup> Furthermore, optineurin interacts with the protein huntingtin, suggesting some role in Huntington's disease,<sup>145</sup> and mutations in optineurin are associated with glaucoma<sup>146</sup> and Paget's disease of the bone.<sup>147</sup> The ubiquilin-1 paralog, with a domain structure similar to ubiquilin-2, is associated with AD.<sup>6</sup> The 14-3-3 protein isoforms co-localized in Cu,ZnSOD inclusions have also been found in a Parkinson's disease model, suggesting some commonalities in inclusion formation.<sup>148</sup> Angiogenin has been implicated in a gamut of diseases, from cancers to diabetes, asthma, and heart disease.<sup>149</sup> Finally, nucleotide repeats (as in *C9ORF72*) are known to cause a variety of neurodegenerative diseases such as Huntington's disease, Fragile X-syndrome, Kennedy's disease and others.<sup>150</sup> These observations underscore the need for meaningful synergistic collaborations among researchers studying these different complex diseases that often involve protein aggregation, allowing new insights to be compounded.

### Treatment of ALS

The primary goal of ALS treatment is the inhibition of disease progression, although an important secondary consideration is the treatment of damage already done. Palliative care (eg, home care and hospice) remains a significant focus of the treatment program for the ALS patient. Non-invasive ventilation, for example, can improve the quality of life and extend survival in non-bulbar patients.<sup>151</sup> A support team, and hospice care toward the end of life can help the ALS

patient to prepare nutritive food that is easy to swallow, provide medications for muscle spasticity, weariness, sleep and depression, and adjust ventilators, enabling the patient to adjust to lifestyle limitations.

Although domestic alterations can provide significant relief to current patients, biochemical and pharmacological advances will drive forward better therapeutics. A panel of ALS biomarkers from non-invasive analyses would be a major gain not only in diagnosis and monitoring progression, but also in identifying affected biological pathways in ALS to target therapeutically.<sup>152</sup> Multiple studies have sought to identify protein biomarkers for ALS, including increased blood or CSF levels of TDP-43, or the cysteine protease inhibitor cystatin C, or a skewed CSF ratio of phospho-neurofilament heavy chain to complement C3.<sup>153–156</sup> Furthermore, the combined efforts of GC/MS (gas chromatography coupled to mass spectrometry), LC/MS (liquid chromatography coupled to mass spectrometry), and NMR (nuclear magnetic resonance) could potentially span the whole metabolome in identifying biomarker signatures.<sup>50,60</sup> Better disease markers could reduce the long duration, averaging 14 months, between initial symptom presentation and diagnosis,<sup>47</sup> helping to improve the disease trajectory.<sup>157</sup> Such endeavors would also provide a platform for personalized medicine for ALS patients. At present, at least one clinical trial (NCT00677768) is being organized to analyze the blood and CSF of ALS patients for biological markers.

## Pharmacological interventions

The only approved medicine to treat the general symptoms of ALS is the anti-excitotoxicity drug riluzole.<sup>158</sup> The drug is thought to preserve motor neuron function by decreasing toxic glutamate levels at glutamatergic nerve terminals by (a) inactivating sodium channels, (b) inhibiting glutamate release, and (c) blocking postsynaptic actions of NMDA receptors.<sup>159</sup> The safety and efficacy profiles for riluzole are better than those for other excitotoxicity drugs, but riluzole only increases the chance of an additional year of survival by about 9%, typically prolonging survival for about 2–3 months.<sup>11</sup> The drug serves to slightly preserve limb and bulbar function but actual muscle strength is typically not improved.<sup>11</sup> Recently approved for treating purely the pseudobulbar affect symptoms less commonly observed in ALS patients is dual-acting dextromethorphan/quinine (sold as Neudexta®; Avanir Pharmaceuticals, Aliso Viejo, CA).<sup>160</sup> Like riluzole, dextromethorphan also inhibits glutamatergic signaling, and quinine helps to increase its bioavailability, providing modest benefit to a subset of patients.<sup>160</sup>

Promising new therapeutic developments, several of which are in late-phase clinical trials, may provide strides forward in treating ALS. One such drug in phase III clinical trials (NCT00349622) is the antibiotic ceftriaxone, used to treat pneumonia and bacterial meningitis. In ALS patients, ceftriaxone appears to upregulate the GLT-1 (EAAT2) glutamate transporter, potentially correcting cellular glutamate levels.<sup>161</sup> Another potential treatment option is high-dose methylcobalamin (vitamine B-12), currently in phase II/III studies (NCT00444613 and NCT00445172) to determine safety and efficacy for long-term use in ALS.<sup>162</sup> This compound was recently shown to reduce homocysteine (another excitatory amino acid)-mediated toxicity in NSC-34 cells.<sup>163</sup> Finally, an antioxidant targeting the mitochondria is currently in phase III trials (NCT01281189), sponsored by Biogen Idec (Weston, MA) and Knopp Biosciences LLC (Pittsburgh, PA). This drug, dextramipexole,<sup>164</sup> is the R(+)-isomer of the amino-benzothiazole drug pramipexole (currently approved to treat Parkinson's disease and restless legs syndrome). Dextramipexole was well tolerated in phase II clinical trials, revealing positive trends in slowing function decline and improving survivability.

## SOD1-targeting therapies

The establishment of mutant *SOD1* transgenic mice in the late 1990s was a major breakthrough in the field, providing the first disease models for ALS.<sup>70</sup> Now, about a dozen such *SOD1* ALS mouse models exist.<sup>165</sup> Other distinctive ALS models have been developed,<sup>166,167</sup> including the newer *TARDBP* mouse models that similarly display ALS-like symptoms such as gait abnormalities, weight loss, and spasticity.<sup>104</sup> However, the use of *SOD1* mouse models has predominated much of the therapeutic progress, in part because *SOD1* represents a major disease target. For example, because the *SOD1* gene is predominately dispensable,<sup>71</sup> reducing its expression and perturbing aggregation are favored strategies for treatment of ALS. These transgenic animals are appropriate models in many cases, and guidelines have been suggested for standardizing studies in *SOD1* mice.<sup>168</sup>

Both small molecules and siRNAs are being explored to downregulate and diminish SOD levels. The hydroxylamine drug arimoclomol (Orphazyme) is currently in stage II/III clinical trials (NCT00706147). This compound induces a heat shock response that resulted in a decrease in ubiquitin-positive aggregates in G93A *SOD1* mouse models,<sup>169</sup> and is now being tested in *SOD1* ALS patients. A free radical scavenger, edaravone (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) was recently found to ameliorate ALS symptoms and diminish SOD aggregate deposition in interior horn cells. Phase III clinical



trials were recently completed (NCT00330681; NCT00424463; NCT00415519), with results pending publication, so the future success of the drug remains to be seen. Studies aimed at silencing *SOD1* using siRNA-based strategies in mice have met with some success,<sup>170,171</sup> although the inability of siRNA to pass the blood–brain barrier makes delivery a problem. Accordingly, Isis Pharmaceuticals Inc (Carlsbad, CA) has developed a CSF-infused delivery method for Isis-SOD1RX antisense oligos that recently were successful in animal models,<sup>172</sup> and are now being examined in phase I clinical trials (NCT01041222). Finally, an approach aimed at prevention, which is in its infancy, is immunization against mutant Cu,ZnSOD through vaccination with mutant Cu,ZnSOD or metal-free Cu,ZnSOD (exhibiting some similar pathogenic properties).<sup>173</sup> As stable Cu,ZnSOD polymers expected to break tolerance exist,<sup>174</sup> and as antibodies favor reactions with more flexible regions,<sup>175,176</sup> such antibody experiments may be promising.

A recent study used patient-derived progenitor cells to derive cultured astrocyte cell lines, and these were found to be toxic to motor neurons, via a mechanism involving secretion of uncharacterized factors. Interestingly, both FALS (mutant *SOD1*) and SALS-derived cells, but not non-ALS derived astrocyte cells, had common pathway changes (namely NF- $\kappa$ B, MAPK, JNK, and AKT), and knockdown of *SOD1* rescued the motor neuron killing phenotype in four of six cell lines examined.<sup>177</sup> This study interestingly reaffirms the use of *SOD1*-targeted therapeutics in the context of SALS (although the effects on other FALS genetic backgrounds were not tested) and also suggests that such cell cultures could prove useful for therapeutic screening in the absence of an all-encompassing ALS disease model. Indeed, a few years ago, astrocyte replenishment by injection of glial precursor cells in *SOD1* model rats was found to prolong life and improve motor performance.<sup>178</sup> Similarly, a phase I clinical trial (NCT01348451) aimed at spinal implantation of spinal cord-derived stem cells is being sponsored by Neuralstem Inc (Rockville, MD). This treatment previously extended the life of *SOD1* transgenic rats by 10 days,<sup>179</sup> and provides the first regenerative medicine strategy for ALS.

## Future directions

Where do we go from here? ALS was first described about 150 years ago<sup>180</sup> and recent biotechnological advances have allowed researchers to begin pinpointing the precise genetics and pathological mechanisms behind the disease. Yet, many questions still remain: How do the distinct pathways involved in the disease overlap and converge to cause similar phenotypes? Can diagnostics improve to the point of early screening and detection? Arguably most importantly, how

can we best treat individual patients? Fortunately, the complex nature of the disease also allows for many potential targets and means for therapeutic intervention.

The discovery of the role of *SOD1* in ALS was a triggering event that significantly advanced our current understanding of the disease aided by the basic science of SOD structure and biochemistry.<sup>87,181</sup> Although we now know that the mutant proteins aggregate, we are only starting to appreciate the key architectural features of the proteins involved in triggering this aggregation and its consequences. More recently, we have realized the significant contributions of TDP-43 and FUS in ALS and other degenerative diseases.<sup>182</sup> Indeed, RNA metabolism appears to be a common thread. The recent identification of ubiquilin-2 as a co-immunolocalized component of ALS inclusions in a wide variety of ALS cell types has also been a major breakthrough in the field.<sup>6</sup> Thus, follow-up work is now needed in order to determine the mechanism of this ubiquilin-mediated pathology, as well as its potential contributions to other ALS-linked pathways. Finally, determining the pathogenic mechanism of action of newly identified *C9ORF72* repeats may prove extremely useful in understanding a significant majority of ALS cases, both sporadic and inherited. Newer disease models will undoubtedly play a significant role in facilitating these studies.

A critical element of progress in the ALS field will be the dissemination of genetic, epidemiologic, and therapeutic information. Fortunately, several helpful online databases and resource are now available, including the ALS online genetics database,<sup>183</sup> the Genetic Association studies website,<sup>184</sup> the ALS forum,<sup>185</sup> and the Northeast ALS Consortium (NEALS).<sup>186</sup> Outreach and social networking is provided by sites such as the Twitter-based ALS Untangled,<sup>187</sup> which hosts a forum for patient conversations. These assets will increase awareness and discourse among ALS patients and drive future research collaborations.

## Conclusions

Currently, ALS is an unrelenting and incurable neuromuscular disease that paralyzes its victims, eventually leaving them incapable of breathing. Gradually, thanks in part due to strides in molecular genetics, the mechanisms leading to aberrant cellular physiology and toxic inclusions are being sewn together. At present, therapeutic strategies aim to slow down the pace of the disease. Ultimately, however, future efforts will work to block the initial events leading to neuronal death. This will prevent damage to the patient's motor ability before it happens, stemming from earlier diagnosis and leading to better prognosis.

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## Disclosure

The authors report no conflicts of interest in this work.

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