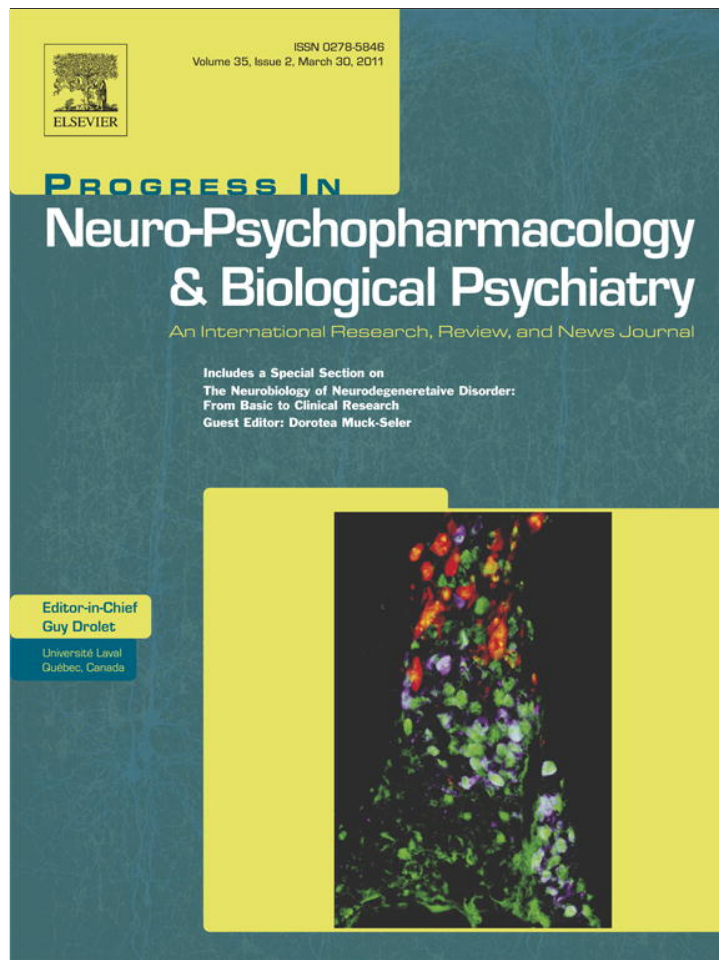




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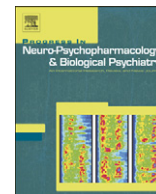
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Molecular basis of amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal disorder of motor neuron degeneration with unclear etiology and no effective treatment to date. ALS is, however, increasingly recognized as a multisystem disorder associated with impaired cognition. The overlap between ALS and dementia at clinical, genetic and neuropathologic levels indicates a spectrum of clinical phenotypes that may include features of frontotemporal lobar degeneration (FTLD). Most cases of ALS are sporadic (SALS), but approximately 10% of all ALS cases are familial ALS (FALS). Mutations in the *Cu/Zn superoxide dismutase-1 gene (SOD-1)* occur in about 20% of FALS cases. Mutations in the *TAR DNA-binding protein 43 gene (TARDBP or TDP-43)* may occur in 3–4% of FALS cases, and less frequently, in FTLD. Recently, mutations in the *fused in sarcoma/translation in liposarcoma gene (FUS/TLS)* were identified as causing about 4–5% of FALS, SALS, and FTLD cases, but not *SOD-1* ALS cases, indicating a pathogenic role of FUS, together with TDP-43, in possibly all types of ALS, except for *SOD-1* linked ALS. TDP-43 and FUS have striking structural and functional similarities, most likely implicating altered RNA processing as a major event in ALS pathogenesis. Thus, *TARDBP* and *FUS/TLS* mutations define a novel class of neurodegenerative diseases called TDP-43- and FUS-proteinopathies, in which both misfolded proteins are novel targets for the development of therapeutics in this spectrum of diseases. However, *SOD-1* linked ALS may have a pathogenic pathway distinct from other types of ALS.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive and ultimately fatal, neurodegenerative disorder caused by degeneration of motor neurons in the brain and spinal cord. The cause is unknown, and no effective treatment currently exists. ALS is used here to describe signs of upper and lower motor neuron degenerations with a progressive spread of symptoms and signs within a region or to other regions, as defined by the *El Escorial* World Federation of Neurology Criteria (Brooks, 1994; Brooks et al., 2000). This review will highlight recent advances in our understanding of the molecular genetics, biochemistry, and neuropathology of ALS, which may facilitate the development of new diagnostic tests and therapeutics.

ALS is increasingly recognized as a multisystem disorder, in which other non-motor impairments can be observed, including cognition (Geser et al., 2008). The cognitive impairment can accompany, or even

precede, the motor symptoms, developing the syndrome of frontotemporal lobar degeneration (FTLD) in ALS (Murphy et al., 2007; Strong et al., 2009), and vice versa FTLD patients often develop motor neuron disease consistent with ALS (Lomen-Hoerth et al., 2002). The presence of frontotemporal impairment in ALS may predict a shorter survival time (Hodges et al., 2003). The impaired cognition, altered in about one-half of all ALS patients, particularly implicate executive dysfunction and mild memory decline in a disease process (Murphy et al., 2007), including deficient performances of verbal fluency (Abrahams et al., 2000; Liscic et al., 2008a).

2. Genetics of ALS

Most cases of ALS are of unknown etiology and appear as sporadic ALS (SALS). About 10% of all ALS cases are familial ALS (FALS). In 1993, Rosen et al. (1993) reported the presence of mutations in the *SOD-1* gene, located on chromosome 21, which encodes the *Cu/Zn superoxide dismutase-1*. Since then, 153 mutations in the *SOD-1* gene have been claimed to be associated with ALS (Andersen, 2006). Mutations in the *SOD-1* gene are found in 12–23% of FALS and 2–3% of SALS (Andersen, 2006). Subsequently, rare genetic defects in several other genes have been found in ALS or ALS-like motor neuron disease (Pasinelli and Brown, 2006). In 2008, Gitcho et al. (2008) and Sreedharan et al. (2008) independently reported that pathogenic mutations in the *TARDBP* gene encoding TAR DNA-binding protein 43 (TDP-43) cause several neurodegenerative diseases such as FALS,

Abbreviations: ALS, Amyotrophic lateral sclerosis; SALS, Sporadic ALS; FALS, Familial ALS; FTLD, Frontotemporal lobar degeneration; FUS/TLS, Fused in sarcoma/translation in liposarcoma; *SOD-1*, Superoxide dismutase-1; TDP-43, TAR DNA-binding protein 43.

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SALS, and FTLD. Their findings support a direct role of *TARDBP* mutations in neurodegeneration. TDP-43 is a 414 amino acid ubiquitously expressed nuclear protein, encoded by *TARDBP* gene located on chromosome 1. It contains two highly conserved RNA-recognition motifs (RRM1 and RRM2), a nuclear localization signal at the N-terminus, and a glycine-rich region mediating protein–protein interactions at the C-terminus (Lagier-Tourenne and Cleveland, 2009). Of the thirty *TARDBP* mutations identified so far, 29 are localized in the highly conserved C-terminal domain of the TDP-43 protein, which is known to be involved in the interaction of the TDP-43 with other proteins. This suggests that *TARDBP* gene mutations may interfere with the normal protein–protein interactions of TDP-43, thus affecting regulation of transcription, RNA splicing and RNA transport (Lagier-Tourenne and Cleveland, 2009) (Fig. 1). Recently, the TDP-43 mutant transgenic mice models with features of ALS and FTLD have been developed in order to define the link between mutations in TDP-43 and ALS, however, with controversial results (Wegorzewska et al., 2009; Wils et al., 2010).

Recently, mutations in the gene encoding fused in sarcoma/translated in liposarcoma (*FUS/TLS*), were identified as a new causal gene for ~4% of FALS (~0.4% of all ALS) (Vance et al., 2009; Kwiatkowski et al., 2009), SALS cases (Corrado et al., 2010; Deng et al., 2010; Lai et al., 2010) and FTLD cases (Van Langenhove et al., 2010), but not in the *SOD-1* ALS cases. Vance et al. (2009) and Kwiatkowski et al. (2009) independently identified 15 different *FUS* mutations in 26 unrelated persons. Most of the mutations were clustered by exons 14 and 15, located in the C-terminal region of the protein. The *FUS/TLS* is a 526 amino acid protein encoded by *FUS/TLS* gene located on chromosome 16. As with TDP-43, *FUS* is a predominantly nuclear protein that is expressed at low levels in the cytoplasm (Lagier-Tourenne and Cleveland, 2009). It also contains glycine-rich region, RNA-recognition motif (RRM), multiple RGG repeats and highly conserved C-terminus showing strong structural similarity with TDP-43 (Fig. 1). Altogether, mutations in specific genes have been identified in about 30% of FALS cases (Deng et al., 2010), with the remaining causes of FALS as yet to be discovered.

3. Neuropathology of ALS

Protein aggregation has been recognized as a pathological hallmark in neurodegenerative disorders (Lansbury and Lashuel, 2006). The neuropathology associated with most ALS cases is characterized by the abnormal accumulation of insoluble proteins in the cytoplasm of degenerating motor neurons (Lowe, 1994; Neumann et al., 2006). Until recently, little was known about the specific biochemical composition of these neuronal cytoplasmic inclusions (NCIs), except that the abnormal protein was ubiquitinated. These ubiquitin immunoreactive NCIs are most common in anterior horn cells (lower motor neurons), accompanied by cortical (upper motor neuron) and brainstem motor neurons. Neuroimmunopathologic studies of TDP-43 place most sporadic and familial cases of ALS within a spectrum of disorders which includes ALS, FTLD, and cases with clinical and neuropathologic features of both ALS-FTLD, (Cairns et al., 2007; Mackenzie et al., 2007; Liscic et al., 2008a,b). In pathological conditions, TDP-43 is abnormally accumulated from the nucleus of neurons and glia cells, to the NCIs. Biochemical analysis of NCIs indicates that pathologic TDP-43 is ubiquitinated, hyperphosphorylated and accumulated as abnormal C-terminally truncated form of 25 kDa (Neumann et al., 2006; Mackenzie and Rademakers, 2008). The neuropathology associated with most FTLD is heterogeneous, characterized by the abnormal accumulation of TDP-43, tau protein or an unidentified ubiquitinated protein, called atypical FTLD-U (Neumann et al., 2009). More than half of all cases of FTLD have cytoplasmic TDP-43 aggregates. Although identification of TDP-43 aggregates proved to be a breakthrough, the pathology alone left it unclear whether aggregation of TDP-43 is a primary event in ALS pathogenesis or whether it is a byproduct of the disease process. As with FALS with *TARDBP* mutation, FALS with *FUS/TLS* mutation is characterized neuropathologically by cytoplasmic aggregates of *FUS*, indicating that *FUS* and TDP-43 inclusions were similar in morphology and distribution. Moreover, it has been shown that *FUS*/TDP-43/ubiquitin positive inclusions are a common feature of the SALS, FALS,

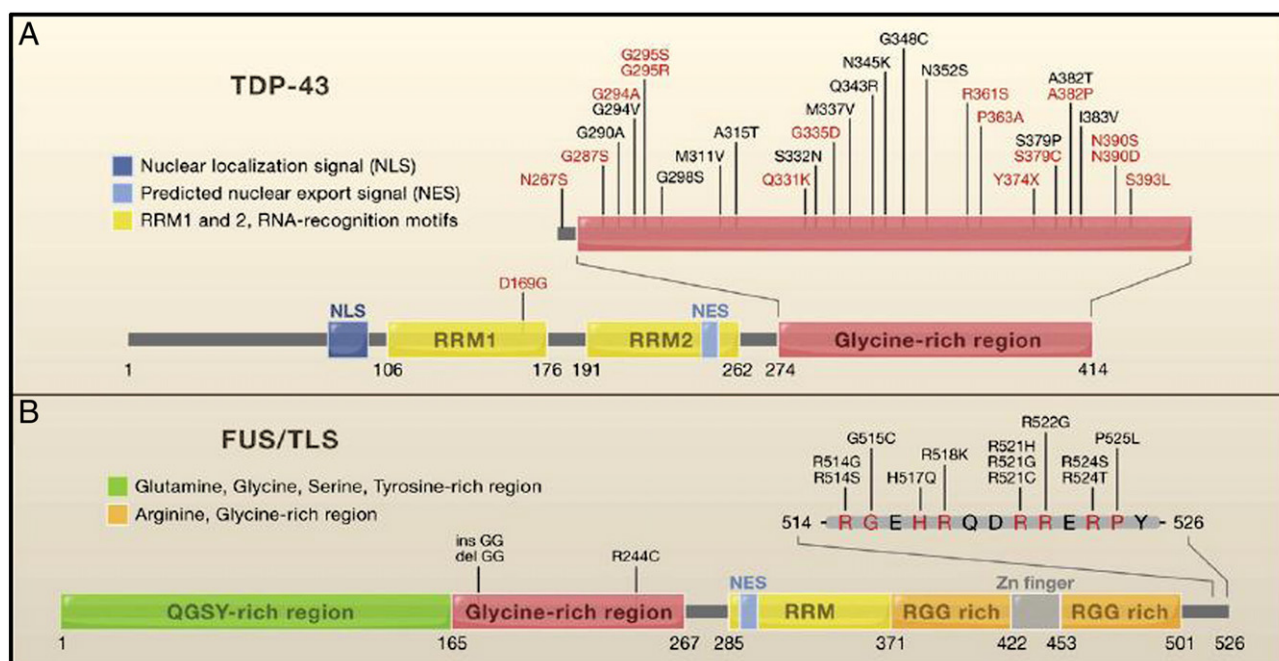


Fig. 1. TDP-43 and FUS/TLS mutations in ALS. (A) Thirty dominant mutations in TDP-43 have been identified in sporadic (red) and familial (black) ALS patients, with most lying in the C-terminal glycine-rich region of TDP-43. All are missense mutations, except for the truncating mutation TDP-43^{Y374K}. (B) Fourteen mutations in FUS/TLS have been identified in familial ALS cases, with most lying in the final 13 amino acids of this protein (R514S and G515S are found in cis). Data compiled from Kwiatkowski et al. (2009); Vance et al. (2009); domains from <http://www.uniprot.org> and <http://www.cbs.dtu.dk/services/NetNES>.

and ALS with dementia (Cairns and Ghoshal, 2010; Deng et al., 2010). However, the absence of pathological TDP-43 and FUS/TLS in FALS harboring *SOD-1* gene mutations implies that motor neuron degeneration may result from a different mechanism in those cases suggesting that the full complement of ALS pathogenic mechanisms is yet to be elucidated (Cairns and Ghoshal, 2010).

The presence of *FUS* mutation in a group of patients with atypical FTLD-U reinforces the concept that FTLD and ALS are closely related conditions (Neumann et al., 2009; Vance et al., 2009). However, the full spectrum of FTLD with FUS pathology remains to be defined and future studies are needed to examine the possible role of FUS also in other types of tau/TDP-43 pathology and vice versa. Screening of additional large cohorts of ALS and FTLD patients is needed to further understand the role of *TDP-43* and *FUS* mutations in the pathogenesis of this group of diseases.

4. Conclusion

ALS and FTLD both are progressive neurodegenerative diseases without known cause (except for *SOD-1*), in the majority of cases, and with no effective disease-modifying treatment to date. Some forms of familial ALS are linked to genetic mutations in specific genes. First identified, mutations in the Cu/Zn *SOD-1* gene are the most common one. Recently, mutations in *TARDBP* and *FUS/TLS* genes were identified as the cause of FALS, SALS and, less frequently, FTLD. TDP-43 and FUS/TLS, structurally and functionally related proteins, regulate several steps of gene expression such as transcription, RNA splicing and RNA transport implicating altered RNA processing as a major event in SALS, FALS and some FTLD, but not *SOD-1* linked ALS. Obviously, *SOD-1* linked ALS appears to have a pathogenic pathway that is independent, at least in part, from other forms of ALS pathogenesis. Recently identified *TARDBP* and *FUS/TLS* mutations define a novel class of neurodegenerative diseases called TDP-43- and FUS-proteinopathies, in which both misfolded proteins are novel targets for the development of new diagnostic tests and therapeutics in this spectrum of diseases.

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References

Abrahams S, Leigh PN, Harvey A, Vythelingum GN, Grise D, Goldstein LH. Verbal fluency and executive dysfunction in amyotrophic lateral sclerosis (ALS). *Neuropsychologia* 2000;38:734–47.

Andersen PM. Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Curr Neurol Neurosci Rep* 2006;6:37–46.

Brooks BR. El Escorial World Federation of neurology criteria for the diagnosis of amyotrophic lateral sclerosis. *J Neurol Sci* 1994;124(Suppl):96–107.

Brooks BR, Miller RG, Swash M, Munsat T. Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1:291–9.

Cairns NJ, Ghoshal N. FUS. A new actor on the frontotemporal lobar degeneration stage. *Neurology* 2010;74:354–6.

Cairns NJ, Bigio EH, Mackenzie IR, Neumann M, Lee VMY, Hatanpaa K, et al. Neuropathologic diagnostic and nozologic criteria for frontotemporal lobar

degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. *Acta Neuropathol* 2007;114:5–22.

Corrado L, Del Bo R, Castellotti B, Ratti A, Cereda C, Penco S, et al. Mutations of FUS gene in sporadic amyotrophic lateral sclerosis. *J Med Genet* 2010;47:190–4.

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.

Geser F, Brandmeir NJ, Kwong LK, Martinez-Lage M, Elman L, McCluskey L, et al. Evidence of multisystem disorder in whole brain map of pathological TDP-43 in amyotrophic lateral sclerosis. *Arch Neurol* 2008;65:636–41.

Gitcho MA, Baloh RH, Chakraverty S, Mayo K, Norton JB, Levitch D, et al. TDP-43 A315T mutation in familial motor neuron disease. *Ann Neurol* 2008;63:535–8.

Hodges JR, Davies R, Xuereb J, Kril J, Halliday G. Survival in frontotemporal dementia. *Neurology* 2003;61:349–54.

Kwiatkowski Jr TJ, Bosco da, Leclerc AD, Tamrazian E, van den Berg CR, Russ C, et al. Mutations in the *FUS/TLS* gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 2009;323:1205–8.

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

Lai SL, Abramzon Y, Schymick JC, Stephan DA, Duncley T, Dillman A, et al. FUS mutations in sporadic amyotrophic lateral sclerosis. *Neurobiol Aging* (published online ahead of print February 4, 2010).

Lansbury PT, Lashuel HA. A century-old debate on protein aggregation and neurodegeneration enters the clinic. *Nature* 2006;443:774–9.

Liscic RM, Stukovnik V, Mück-Seler D, Babic A, Nedic G, Mustapic M, et al. Cognitive changes and genetic markers in amyotrophic lateral sclerosis: preliminary results of a prospective study. *Dement Geriatr Cogn Disord* 2008a;26(Suppl. 1):64–5.

Liscic RM, Tenenholz Grinberg L, Zidar J, Gitcho MA, Cairns NJ. ALS and FTLD. Two faces of TDP-43 proteinopathy. *Eur J Neurol* 2008b;15:772–80.

Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology* 2002;59:1077–9.

Lowe J. New pathological findings in amyotrophic lateral sclerosis. *J Neurol Sci* 1994;124(Suppl):38–51.

Mackenzie IR, Rademakers R. The role of transactive response DNA-binding protein-43 in amyotrophic lateral sclerosis and frontotemporal dementia. *Curr Opin Neurol* 2008;21:693–700.

Mackenzie IR, Bigio EH, Ince PG, Geser F, Neumann M, Cairns NJ, et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol* 2007;61:427–34.

Murphy JM, Henry RG, Langmore S, Kramer JH, Miller BL, Lomen-Hoerth C. Continuum of frontal lobe impairment in amyotrophic lateral sclerosis. *Arch Neurol* 2007;64:530–4.

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006;314:130–3.

Neumann M, Rademakers R, Roeber S, Baker M, Kretschmar HA, Mackenzie IRA. A new subtype of frontotemporal lobar degeneration with FUS pathology. *Brain* 2009;132:2922–31.

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

Rosen DR, Siddique T, Paterson 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.

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

Strong MJ, Grace GM, Freedman M, Loman-Hoerth C, Woolley S, Goldstein LH, et al. Consensus criteria for the diagnosis of frontotemporal cognitive and behavioral syndromes in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2009;10:131–46.

Van Langenhove T, van der Zee J, Sleegers K, Engelborghs S, Vandenberghe R, Gijssels I, et al. Genetic contribution of FUS to frontotemporal lobar degeneration. *Neurology* 2010;74:366–71.

Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 2009;323:1208–11.

Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH. TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. *PNAS* 2009;106(44):18809–14.

Wils H, Kleinberger G, Janssens J, Pereson S, Joris G, Cuijt I, et al. TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. *PNAS* 2010;107(8):3858–63.