

Pathogenic *TARDBP* mutations in amyotrophic lateral sclerosis and frontotemporal dementia: disease-associated pathways

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Synopsis

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are both late-onset neurodegenerative disorders that are associated with mutations in *TARDBP*. The product of this gene, TDP-43, has also been identified as the main component of intracellular inclusions typical of most cases of ALS and FTD. Recent evidence suggests that TDP-43 is essential for proper development and is involved in several fundamental cellular processes, including gene transcription, RNA processing, and the spatial regulation of mRNA translation. Pathogenic *TARDBP* mutations that impair TDP-43 function could therefore be related to neuronal degeneration in ALS and FTD. Conversely, cellular and animal studies have shown that pathogenic *TARDBP* mutations induce neuronal toxicity through mislocalization or elevated concentrations of TDP-43, consistent with a gain-of-function mechanism. In this review, we focus upon the physiologic functions of TDP-43 within the central nervous system and discuss how these functions may be perturbed or pathologically altered by disease-associated mutations.

Neurodegenerative diseases are a growing problem for the elderly /1/. Age is the most important risk factor for this group of disorders, and as more of the population reaches advanced ages, increasing numbers of people succumb to frontotemporal dementia (FTD) /2,3/, amyotrophic lateral sclerosis (ALS) /4,5/, and other neurodegenerative conditions /1,6/. While few effective therapies for these disorders exist, our understanding of the molecular events involved in disease pathogenesis has matured considerably in the past several years.

In this review, we will explore the link between ALS and FTD and how recent investigations into this association have radically changed our conceptions of these conditions. In particular, we will focus upon the significance of a single protein, TDP-43, in the development of ALS and FTD, and the ways in which this protein might contribute to the specific clinical and pathologic features of disease.

The clinical impact of ALS

ALS is a relentlessly progressive neurologic disorder that manifests clinically with the gradual and widespread development of weakness and spasticity /4,7/. The peak incidence is between the ages of 40 and 70, and the median time from symptom onset to death, often due to respiratory muscle involvement, is 30–36 months /8/. The incidence of ALS is remarkably similar throughout the world, affecting 1.5–2.5 patients per 100,000 individuals /4,5/. Although medical advances over the past two decades have substantially prolonged the survival of patients with ALS, they have had no appreciable effect upon the rate of functional decline in those afflicted /9/. The early use of non-invasive ventilation /10/ and nutritional support /11/, improved care within hospitals /12/, and multidisciplinary clinics /13/ all contribute to prolonged survival but fail to significantly improve the course of the disease itself.

ALS is a clinically and genetically heterogeneous disorder. Patients may present with predominantly upper motor neuron loss, lower motor neuron degeneration, or a combination of the two /8,14/. Some have early bulbar involvement, but in others, the muscles of the head and neck may be completely spared throughout the disease course. Moreover, growing evidence indicates that ALS is a multisystem disorder, affecting areas of the frontal and temporal cortices, brainstem, and cerebellum, in addition to the traditionally recognized motor system involvement /15-17/. More than 14 genes are associated with familial ALS (fALS), which accounts for approximately 10% of all diagnoses /18-22/. Mutations in the gene encoding superoxide dismutase-1 (SOD1)

are the most common cause of fALS, responsible for 20% of fALS or 2% of all cases. Animal models of ALS, such as transgenic mice and rats expressing mutant SOD1 (mSOD1), successfully recapitulate the clinical and pathologic features of motor neuron disease in humans (reviewed in references /23,24/) and have led to development of several promising treatments that slow disease onset and progression in animals. Still, despite more than 30 clinical trials of compounds and strategies that were effective in transgenic mSOD1 mice, none has showed any significant benefit in humans with ALS /24,25/. In fact, the only medication with demonstrated efficacy in humans, riluzole, was developed independently of transgenic mSOD1 animals /26,27/.

The fundamental connection between ALS and FTD

As initially described by Jean-Martin Charcot in 1869, ALS is a pure motor disorder that spares cognition /8,14/. However, on detailed neuropsychiatric testing, up to 1/3 of patients with ALS demonstrate subtle behavioral and personality deficits consistent with FTD, and 15% meet clinical criteria for the diagnosis of FTD /28,29/. FTD is the second most common form of dementia affecting individuals under the age of 65 /2,3/ and can be clinically distinguished from other neurodegenerative conditions by characteristic behavioral, personality, and language abnormalities with relative preservation of episodic memory /3,30,31/. Voxel-based MRI provides an anatomic correlate to the clinical symptoms exhibited by these patients, revealing substantial atrophy in areas of the frontal and temporal cortices responsible for language and the regulation of social behavior /32-34/. Just as patients with ALS often develop signs of FTD, approximately 15% of patients with FTD suffer from a motor neuron disease (MND) that is clinically and pathologically indistinguishable from sporadic ALS (sALS) /35-38/. In a subset of FTD patients without demonstratable signs of MND, ubiquitin-rich deposits are sometimes found in the neurons of the motor cortex and anterior horn of the spinal cord /38/. These findings suggest the presence of sub-clinical pathology in patients without obvious symptoms of disease.

Over 40% of patients with FTD have one or more family members affected by the disorder, consistent with a strong genetic contribution to the pathogenesis of FTD /39,40/. Mutations in the gene encoding progranulin (*GRN*) account for approximately 50% of the inheritance of FTD, while mutations in the microtubule-associated protein tau gene (*MAPT*) are responsible for an additional 25% of cases. FTD is characterized pathologically

by protein-rich deposits within neurons and surrounding glia, spongiform degeneration, neuronal loss and atrophy. These are frequently found within areas of the frontal and temporal cortices that correspond closely to functional deficits on clinical examination /41-44/. For example, behavioral variant FTD is marked by prominent volume loss within the dorsal and medial frontal lobes, while language-predominant FTD subtypes (semantic dementia or primary progressive aphasia) feature more severe atrophy of the bilateral inferior temporal lobes or left insula /42,43,45/. The pathologic classification of FTD relies upon the identification of the principal protein component of the neuronal and glial inclusions typical of the disorder¹. Frontotemporal lobar dementia (FTLD) with deposits rich in TDP-43 (transactive response element DNA-binding protein of 43 kDa, FTLD-TDP) and ubiquitin is the most common type of pathology noted on post-mortem examination: it is found in over 50% of cases that go on to post-mortem examination. Inclusions that demonstrate strong staining with antibodies directed against tau (FTLD-tau) account for approximately 40% of the total, and protein aggregates containing the FUS/TLS (fused in sarcoma/translated in liposarcoma, FTLD-FUS) protein are noted in less than 10% /41,46,47/.

The clinical similarities between FTD and ALS suggested that these disorders represent opposite ends of a spectrum of disease /17,48,49/, a hypothesis that has since been confirmed by pathologic and biochemical studies. In 2006, two separate groups identified TDP-43 as the major protein constituent of intracellular inclusions characteristic of sALS and the majority of fALS and FTLD /50,51/. TDP-43 is universally expressed and localized predominantly in the nucleus in healthy cells /52/. However, in affected neurons from patients with ALS or FTLD-TDP, the protein characteristically redistributes from the nucleus to the cytoplasm, where it accumulates in detergent-insoluble aggregates /50,51,53,54/. Unlike its nuclear counterpart, TDP-43 recovered from cytoplasmic inclusions is hyperphosphorylated and truncated at the amino-terminus /54-57/. Interestingly, humans with fALS associated with mutant SOD1 and transgenic mSOD1 animals show no signs of abnormal TDP-43 accumulation or post-translational modification /37,58-62/. These observations suggest that SOD1 mutations produce motor neuron disease through unique molecular pathways, and may help explain why

¹ In the United States, the term FTD has been reserved for clinical diagnosis only, referring to patients with behavioral or language variants of the disorder. The pathologic correlate to FTD is frontotemporal lobar dementia or FTLD. However, the terminology can be misleading. Physicians in Europe often reserve FTD for the behavioral variant of the disorder and use FTLD to describe all other clinical and pathologic variations.

treatments developed with the use of transgenic mSOD1 models failed to demonstrate substantial efficacy in human trials /24/.

TDP-43 is directly involved in the development of ALS and FTLD

The identification of TDP-43 within neuronal and glial inclusions typical of the majority of ALS and FTLD cases /50,51/ provided the first evidence that this protein is important in disease pathogenesis. Further confirmation emerged from genetic studies demonstrating that mutations in the gene encoding TDP-43 (*TARDBP*) on chromosome 1 result in dominantly inherited ALS and FTD /59,63-77/. To date, over 30 pathogenic mutations have been identified within *TARDBP*. All are associated with MND, and a number with FTD-MND (Figure 1 and Table). In addition, several *TARDBP* mutations have been noted in individuals with ALS without a family history of the disorder, suggesting that *de novo* mutations in *TARDBP* contribute to sporadic-onset ALS as well. Subsequent studies confirmed that *TARDBP* mutations account for 4–6% of fALS and 0.5–2% of all ALS diagnoses /78-80/. Mutations linked to an unidentified locus on chromosome 9 are associated with FTD, ALS, or FTD-MND in affected family members, all of whom demonstrate TDP43-rich neuronal deposits on pathology /81-83/. Moreover, FTD due to mutations in *GRN* /84-87/ and the gene encoding valosin-containing protein (*VCP*) /88,89/ both result in FTLD with intraneuronal deposits containing TDP-43. Thus, mutations in *TARDBP*, *GRN*, *VCP*, and a locus on chromosome 9 all lead to a selective clinicopathologic syndrome characterized by TDP-43 deposition and neuronal loss within the frontal and temporal cortices or the corticospinal tract, ultimately causing cognitive dysfunction or motor symptoms consistent with FTD, ALS, or both.

These disorders are collectively referred to as TDP43-proteinopathies because their distinct molecular pathology centers around the abnormal deposition of protein aggregates rich in TDP-43 /16,17,21,48,90/. The unique clinical symptoms in each case are closely related to the specific pattern and anatomic distribution of pathology, selectively affecting particular neuronal subtypes or regions of the central nervous system. For instance, *GRN* mutations result in frontal and temporal cortical atrophy and FTD, but motor neuron pathology and ALS-like symptoms are rare /91/. In contrast, *TARDBP* mutations induce more widespread pathology with preferential degeneration of the corticospinal tract, producing symptoms of ALS and, less commonly, FTD /80/. A discussion of the specific factors contributing to the selective vulnerability of neuronal subtypes in inherited

neurodegenerative disorders is beyond the scope of this review, but we refer the reader to recent reviews for more information /92-95/.

Several neurodegenerative conditions are characterized by mutations within disease-associated proteins that are integrally involved in disease pathogenesis (reviewed in /6/). Likewise, the importance of TDP-43 in the development of ALS and FTD has been confirmed by the identification of *TARDBP* mutations in individuals with familial motor neuron disease and/or dementia /59,63-77/. As mentioned previously, in dead and dying neurons TDP-43 is redistributed from the nucleus, where it is normally concentrated, to the cytoplasm /50,51/. Cytoplasmic TDP-43 accumulates in detergent-insoluble aggregates, much like those found in Alzheimer's disease, Parkinson's disease, and prion disorders /96,97/. Furthermore, cytoplasmic TDP-43 is hyperphosphorylated /53-55/, similar to tau in the neurofibrillary tangles typical of Alzheimer's disease /98/ and the tauopathies /99/, and subject to partial proteolysis, as seen with prion-protein deposits characteristic of prion diseases /100/. Overexpression of TDP-43 results in cellular toxicity in several model systems, including yeast /101/, neuroblastoma cells /102,103/, and primary cortical /104/ and motor neurons /105/. Furthermore, zebrafish /105/ and rodents /106-109/ develop signs and symptoms of motor neuron disease upon high-level expression of wild-type (WT) or mutant TDP-43. In humans, pathogenic mutations within the 3' untranslated region (UTR) of *TARDBP* result in a twofold higher TDP-43 mRNA and protein levels than those found in controls /69/. These results strongly suggest that abnormal TDP-43 expression or function is intimately connected to disease pathogenesis in ALS or FTD, but the precise cellular events responsible for disease onset and progression are unclear.

TDP-43 function and dysfunction

A thorough understanding of the structure and physiologic function of TDP-43 is essential for determining how mutations in *TARDBP* lead to neurodegeneration. TDP-43 was initially identified as an antiviral factor that prevents HIV replication by directly associating with and inhibiting the HIV transactive response element /110/. *TARDBP*, located on chromosome 1, is expressed in all cell types and tissues, except for cardiac myocytes /52,111/. TDP-43 is a highly conserved protein with significant homology to heterogeneous ribonuclearprotein (hnRNP) family members /112,113/, many of which function in nucleic acid metabolism. TDP-43 contains two

separate RNA recognition motifs (RRM1 and 2) that bind to UG and TG repeats in RNA and DNA, and a glycine-rich domain (GRD) that is important for protein-protein interactions. In particular, the GRD is essential for the association between TDP-43 and other hnRNP family members, including hnRNP A1, A2/B1 and A3 /52,112,113/. All but two of the pathogenic *TARDBP* mutations are clustered within the GRD (Figure 1 and Table), suggesting that this domain is central to disease pathogenesis /114/.

TDP-43 is a DNA- and RNA-binding protein with several functions in the nucleus that might be important for disease pathogenesis (Figure 2). Through its interactions with single-stranded regions of DNA rich in pyrimidines, TDP-43 inhibits or enhances gene transcription, as demonstrated by the protein's effect upon the HIV transactive response element and murine SP-10 promoter, respectively /110,112,115/. Moreover, TDP-43 is required for the proper splicing of mRNA encoding the cystic fibrosis membrane conductor receptor /52,116/, apolipoprotein A-II /117/, and survival of motor neuron (SMN) /118/. Together with SMN, TDP-43 contributes to the formation of nuclear bodies, foci of active gene transcription and RNA splicing within nuclei /119/. Duplications or deletions involving the SMN1 gene cause spinal muscular atrophy in humans /120/, suggesting that TDP-43 dysfunction might induce motor neuron loss through improper splicing of SMN1 pre-mRNA or disruption of the internal structure of nuclear bodies. TDP-43 also associates with elements of the RNA microprocessor complex, including Drosha /121/, and directly binds to and modifies the half-life of low molecular weight neurofilament (NFL) mRNA /122,123/. Abnormal regulation of NFL mRNA levels is common in humans with ALS /124/, and disruption of NFL mRNA stoichiometry produces motor neuron loss and symptoms of ALS in animal models /125,126/. Thus, pathogenic mutations in *TARDBP* might cause motor neuron degeneration by interfering with the normal processing and regulation of neurofilament mRNA /127/.

At steady state, the majority of TDP-43 in healthy cells is confined to the nucleus /50,51/, commensurate with the protein's purported roles in RNA metabolism. However, TDP-43 is not a static protein and shuttles continuously between the nucleus and cytoplasm /128,129/. It contains a nuclear localization signal (NLS) and a nuclear export signal /129,130/, facilitating the transport of the protein and its cargo to and from the nucleus. Within the cytoplasm of healthy cells, TDP-43 is found in small granules that transport, sequester, and degrade mRNA species /131/. These granules co-localize with components of RNA stress granules, including TIA-1 /132-134/, as well as markers of RNA processing bodies, such as Staufen-1 and eIF4E /132,133,135/.

indicating that TDP-43 actively participates in mRNA trafficking and stabilization. The presence of TDP-43 within RNA granules also implies that this protein is involved in the spatial and temporal regulation of mRNA translation, a process that is essential for proper neuronal development, plasticity, and regeneration after injury (reviewed in reference /136/).

Accumulating evidence suggests that the subcellular localization of TDP-43 is tightly controlled and that maintaining TDP-43 localization is crucial for neuronal health. In axotomized neurons, TDP-43 mRNA and protein levels increase, and the protein is redistributed from the nucleus to the cytoplasm, where it transiently accumulates /133,137/. Within 28 days of injury, however, TDP-43 expression returns to baseline levels, and the protein re-accumulates within the nucleus, suggesting that upregulation and cytoplasmic redistribution of TDP-43 could be involved in the response to neuronal injury /133,137/. In neurons isolated from transgenic animals lacking NFL, TDP-43 accumulates within the cytoplasm after axotomy, but the normal distribution cannot be reestablished, and these cells eventually undergo apoptosis /138/. A similar redistribution of TDP-43 from the nucleus to the cytoplasm has been noted in dead and dying neurons from the brains of patients with ALS or FTD /50,51/. Furthermore, expression of cytoplasmic TDP-43, created by disrupting the protein's NLS, is toxic to rodent primary cortical neurons /104/. These findings suggest that cytoplasmic redistribution of TDP-43 initially represents a coping response to injury, perhaps facilitating neuronal recovery, but lead to neurotoxicity and cellular degeneration if the proper distribution cannot be reestablished. In agreement with this hypothesis, cytoplasmic TDP-43 inclusions colocalize with phosphorylated Smad proteins /139/, which mediate intracellular signal transduction through the transforming growth factor-beta (TGF- β) pathway, vital for the maintenance of neuronal health. Persistent cytoplasmic accumulation of TDP-43 might irreversibly sequester Smad proteins and induce toxicity by inhibiting downstream targets of TGF- β that are essential for neuronal survival /140/.

Loss-of-function mechanisms in TDP43-proteinopathies

If fully functional TDP-43 is indeed critical for neuronal sustenance, then mutations that disrupt its level or function within the nucleus or cytoplasm should recapitulate features of disease. Several studies suggest that the amount of TDP-43 is highly regulated and the presence of the protein is necessary for proper development

and survival. Importantly, transgenic mice harboring null mutations in *TARDBP* do not survive past embryonic day 6 /141,142/. TDP-43 can be detected within the nucleus of both wild-type (WT) and null-mutant embryos until embryonic day 2.5, likely reflecting the presence of maternal TDP-43 carried over from the fertilized zygote. Although WT and null-mutant embryos are indistinguishable at this stage, *TARDBP*^{-/-} embryos fail to develop an inner cell mass and die shortly afterwards. Interestingly, homozygous *TARDBP*^{+/+} and heterozygous *TARDBP*^{+/-} embryos exhibit identical TDP-43 mRNA and protein levels, suggesting that the amount and function of TDP-43 are precisely regulated in developing embryos and essential for vitality /141,142/. Additional studies confirmed the toxic effect of TDP-43 downregulation in neuroblastoma cells *in vitro* /143/. Moreover, in *Drosophila* /144,145/ and zebrafish /105/ TDP-43 knockout induces an MND-like phenotype characterized by impaired locomotion and feeding. Knockout animals have impaired sensory /146/ and motor neuron branching /144/, with disorganization and shortening of motor neuron axons /105/. TDP-43 is therefore necessary for embryonic development and appropriate coordination of motor neuron axon outgrowth.

Do pathogenic *TARDBP* mutations negatively affect the function of TDP-43 and result in disease through loss-of-function mechanisms? Potential mechanisms by which TDP-43 dysfunction results in neurodegeneration are shown in Figure 2. In this scenario, mutant TDP-43 cannot carry out the essential functions of WT TDP-43, and the autosomal dominant pattern of inheritance associated with *TARDBP* mutations could be due to either haploinsufficiency or a dominant-negative effect of the mutant protein. There is precedence for both mechanisms in the development of inherited neurodegenerative conditions. For instance, mutations in the genes encoding VCP /88,89/ or charged multivesicular body protein 2B (CHMP2B) /148/, which is also associated with autosomal-dominantly inherited FTD, induce the formation of non-functional autophagosomes that effectively sequester WT proteins. These findings suggest that neurodegeneration could be triggered through a dominant-negative mechanism /149-151/. On the other hand, most pathogenic mutations in *GRN* that result in autosomal-dominant familial FTD decrease the stability of progranulin-encoding mRNA by enhancement of nonsense-mediated decay, thereby resulting in inadequate levels of progranulin (haploinsufficiency) /85,87,147/.

In support of a haploinsufficiency model, several studies demonstrated that pathogenic mutations in *TARDBP* disrupt the physiologic function of the protein. Unlike WT TDP-43, mutant TDP-43 cannot rescue the

locomotor phenotype in zebrafish lacking endogenous TDP-43 /105/. In *Drosophila* sensory neurons, TDP-43 knockdown impairs dendritic branching, an effect that can be fully restored by expression of WT but not mutant TDP-43 /146/. Furthermore, while WT TDP-43 induces the expression of histone deacetylase 6 (HDAC6) in transformed cell lines, mutant TDP-43 had no such effect /143/. These observations suggest that mutant TDP-43 cannot functionally compensate for the WT protein. As discussed above, TDP-43 is involved in several fundamental cellular processes, including the regulation of spatiotemporal mRNA translation, neurofilament mRNA stoichiometry, and mRNA splicing. Disruption of any one of these pathways singularly or in combination could result in cellular dysfunction and death (Figure 2).

Alternatively, *TARDBP* mutations might lead to neurodegeneration through a dominant-negative, loss-of-function mechanism. Mutant TDP-43 accumulates within the cytoplasm of transfected primary neurons /104/, suggesting that *TARDBP* mutations can affect the subcellular distribution of the protein. Interestingly, cytoplasmically targeted TDP-43 sequesters full-length, endogenous TDP-43 within cytoplasmic inclusions /130,132/, and nuclear TDP-43 is depleted by expression of carboxy-terminal TDP-43 fragments that accumulate within the cytoplasm /152/. Since TDP-43 dimerizes *in vitro* and *in vivo* /153/, mutant TDP-43 or C-terminally truncated TDP-43 might reduce nuclear protein levels by dimerizing with the WT, full-length protein and sequestering it within the cytoplasm /153/. These results suggest that mutant TDP-43 or C-terminal fragments of TDP-43 act in a dominant-negative manner by limiting the nuclear import of WT TDP-43. If the presence of TDP-43 within the nucleus is required for some or all of its essential functions, then the forced redistribution of TDP-43 from the nucleus to the cytoplasm might be directly toxic to neurons.

Gain-of-function toxicity in TDP43-dependent neurodegeneration

In many cases, disease-associated mutations result in cellular dysfunction by bestowing novel and potentially deleterious functions on the gene product. Toxic gain-of-function mutations are not uncommon in dominantly inherited neurodegenerative conditions and might be important in spinocerebellar ataxia /154/, prion disorders /155/, Huntington's disease /156/, and ALS /157/. Over 100 pathogenic mutations have been identified within the *SOD1* gene, and with few exceptions, they are all dominantly inherited (see <http://alsod.iop.kcl.ac.uk/> for a full list of mutations). The pathogenic effect of *SOD1* mutations is completely independent of the dismutase

activity of the SOD1 protein: transgenic animals expressing mutant SOD1 with or without dismutase activity develop an identical motor neuron disease phenotype (reviewed in /23,92/). In these animals and in humans carrying *SOD1* mutations, disease is the result of a toxic gain of function of the SOD1 protein. Often, disease-specific features can be recapitulated by overexpressing the disease-associated protein itself, as occurs naturally with gene duplications or triplications. In individuals with Down syndrome or trisomy 21, for instance, early-onset Alzheimer's disease is due to duplication of the gene encoding the amyloid precursor protein on chromosome 21 /158,159/, and triplication of the alpha-synuclein locus has been linked to autosomal dominant, familial Parkinson's disease in humans /160/.

TARDBP mutations might cause fALS and FTD through identical toxic gain-of-function mechanisms. Overexpressing WT or mutant TDP-43 induces cellular toxicity in several experimental systems, including yeast /101/, primary neurons /104,105/, rodents /106-109/, and *Drosophila* /145,161/. Intriguingly, mutations within the non-coding regions of the *TARDBP* locus might result in high-level expression of TDP-43 in humans and the subsequent development of neurodegenerative disease /67,69/. Furthermore, TDP-43 accumulates within the epidermis of individuals with spontaneous ALS, but not age-matched controls /162/, suggesting that elevated TDP-43 protein levels are correlated with both inherited and spontaneous disease. Using quantitative fluorescence microscopy, we showed that the toxicity of TDP-43 is dose-dependent in primary cortical neurons /104/, an effect that was confirmed in transgenic animals expressing increasing amounts of TDP-43 /107,109/. Furthermore, overexpressing exogenous TDP-43 in each case induces neurodegeneration in cells or animals that make endogenous TDP-43. Therefore, at high concentrations, TDP-43 is toxic and exhibits lethal gain-of-function properties.

Importantly, overexpressing TDP-43 in animals induces a neurodegenerative condition that closely resembles ALS and FTD in humans. Transgenic mice expressing WT /107/ or mutant TDP-43 carrying the A315T mutation /108/ develop progressive spasticity, stumbling gait, abnormal reflexes, and weakness culminating in paralysis and death within weeks. Remarkably, the pathology of these animals is limited to the ventral horn of the spinal cord and layer 5 of the neocortex, including the primary motor and somatosensory areas. Astrocytosis, neuronal degeneration and intracellular cytoplasmic aggregates rich in ubiquitin /108/ and TDP-43 /107/ feature prominently in these regions, reminiscent of the selective pathology noted in humans with

TDP43-proteinopathies /48,90/. In addition, constitutive or tetracycline-regulated expression of mutant TDP-43 in transgenic rats induces rapid-onset paralysis and eventually death in affected animals. These symptoms are accompanied by the early loss of motor neuron axon terminals, degeneration of neuromuscular endplates, and during late stages of disease, motor neuron apoptosis, astrocytosis, and microgliosis /109/. These observations show that overexpression of TDP-43 in transgenic animals produces a specific type and distribution of neuropathology limited to predefined areas of the neocortex and spinal cord, much like that seen in humans with FTD and ALS.

How might pathogenic *TARDBP* mutations result in a toxic gain-of-function phenotype? Some possibilities are depicted in Figure 2. As mentioned above, cytoplasmic accumulation of TDP-43 is a fundamental characteristic of ALS and FTLD-TDP /41,48/, and *TARDBP* mutations induce cytoplasmic mislocalization of the mutant protein /104/. Cytoplasmic aggregation of TDP-43 could sequester functional TDP-43 from its predominant site of action, the nucleus, causing toxicity through a loss-of-function mechanism. However, cytoplasmic TDP-43 might also have toxic properties independent of nuclear TDP-43, generating lethal downstream effects through a gain-of-function pathway. The two possibilities are not mutually exclusive, and both might operate simultaneously to produce the neurodegeneration characteristic of TDP43-proteinopathies.

Evidence supporting the direct toxicity of cytoplasmic TDP-43 has emerged from studies of primary cortical neurons expressing mutant TDP-43 /104/. The expression of mutant, but not WT, TDP-43 was lethal to primary cortical neurons, and the toxicity of the mutant protein depended heavily on the subcellular localization of TDP-43. In these experiments, cytoplasmic accumulation of TDP-43 was significantly more common in neurons expressing mutant TDP-43, than in those expressing WT protein. Surprisingly, neurons that exhibited purely nuclear distribution of mutant TDP-43 were protected from the toxicity, and targeting of WT TDP-43 to the cytoplasm by disruption of the NLS recapitulated the toxicity of the mutant protein. Moreover, the toxic effect of cytoplasmic TDP-43 was dose-dependent, while the amount of nuclear TDP-43 was unrelated to neuronal survival, consistent with a gain-of-function toxicity of cytoplasmic TDP-43 /104/. These results strongly suggest that mutations in *TARDBP* cause neurodegeneration by inducing the accumulation of cytoplasmic TDP-43, which may induce cellular toxicity through downstream mechanisms that compromise neuronal survival.

The molecular pathways by which cytoplasmic TDP-43 acts are unknown, but may include aberrant association with cytoplasmic mRNA or RNA-binding proteins, sequestration of nuclear import or export factors, impairment of translation, or proteasome inhibition (Figure 2). TDP-43 interacts with multiple cytoplasmic proteins, including many ribosomal subunits and elongation factors essential for proper mRNA translation /135/. Therefore, cytoplasmic TDP-43 might induce neurotoxicity by abnormal interaction with these components, thereby preventing constitutive translation of proteins essential for the maintenance of neuronal survival or enhancing the translation of potentially deleterious proteins. Disruptions in the cytoplasmic regulation of protein translation contribute significantly to disease pathogenesis in neuromuscular disorders, such as myotonic dystrophy (reviewed in /163/), suggesting that a similar process could be responsible for the neuronal loss characteristic of TDP43-proteinopathies.

Long-lived and metabolically active cells, such as neurons, rely on efficient protein turnover mechanisms to prevent abnormal protein deposition and aggregation. The ubiquitin proteasome system (UPS) mediates the targeted degradation of misfolded and aggregated neuronal proteins, and disruption of the UPS is a fundamental feature of several neurodegenerative conditions /164/. TDP-43 binds to ubiquilin-1, one of the key proteins involved in the specific targeting of proteins for destruction by the UPS /165/. Pathogenic *TARDBP* mutations might induce neurotoxicity by disrupting the association between ubiquilin-1 and TDP-43 and interfering with UPS function. Surprisingly, expression of ubiquilin-1 induces a decrease in TDP-43 protein levels, suggesting that ubiquilin-1 facilitates TDP-43 degradation by the UPS /161/. If mutations in *TARDBP* prevent ubiquilin-1 from binding to and targeting TDP-43 for destruction, mutant TDP-43 might escape degradation and accumulate to lethal concentrations within the cytoplasm of neurons.

A pathologic hallmark of neurodegenerative conditions is the deposition of disease-specific proteins in insoluble aggregates /96,97/. These inclusions are most often found in conjunction with dead or degenerating neurons, an observation that initially led investigators to believe that the aggregates are themselves pathologic. However, more recent evidence suggests that neuronal inclusion body formation is a functional coping response to cellular stress in neurodegenerative diseases and that the inclusions might not be toxic *per se* /166-170/. By sequestering soluble disease-associated proteins into insoluble aggregates, the process of

inclusion body formation could prevent the respective proteins from inducing deleterious downstream effects and limit their toxicity.

How might inclusion body formation be involved in the pathogenesis of TDP-43 proteinopathies? Disease-associated mutations in TDP-43 shorten the time between inclusion body formation and death in a yeast model, indicating that TDP-43 accumulation and aggregation are toxic [101,171]. In contrast, however, overexpression of mutant and WT TDP-43 within primary neurons leads to the formation of inclusion bodies, but inclusion body formation does not significantly affect neuronal survival and is unrelated to the toxicity of mutant TDP-43 [104]. This suggests that inclusion body formation is unnecessary for mutant TDP-43-specific toxicity in neurons. In support of this conclusion, TDP-43 immunopositive inclusion bodies were detected in neurons from transgenic mice overexpressing WT [107], but not mutant TDP-43 [108]. Conversely, transgenic rats [106,109] and mice [108] expressing mutant TDP-43 develop rapidly progressive paralysis with profound degeneration and loss of motor neurons within the spinal cord, in the absence of inclusion bodies containing TDP-43. If inclusion body formation were important or necessary for motor neuron loss, intracellular aggregates rich in TDP-43 should be an early finding, preceding motor neuron dysfunction or death. However, insoluble and ubiquitinated degradation products of mutant TDP-43 were detected in the spinal cords of late-stage transgenic rats only [109]. Thus, soluble mutant TDP-43 is likely to be the toxic molecule in these animals, while inclusion body formation might be a secondary process exhibited by neurons under stress. Indeed, levels of diffuse forms of TDP-43 in neurons significantly predicted whether and when neurons would undergo degeneration [104]. In humans, a single anatomic study demonstrated that the frequency neuronophagia, the engulfment of neurons by activated macrophages and a central feature of ALS pathology, is unaffected by the presence of intracellular TDP-43 inclusions [172]. Inclusion body formation did not appear to provide a survival benefit in this instance, but further investigations involving the longitudinal analysis of motor neuron survival are warranted to conclusively determine whether or not inclusion body formation protects neurons from the toxic effects of TDP-43.

Familial ALS and FTD have also been linked to pathogenic mutations in the gene encoding FUS/TLS, another RNA-binding protein with substantial structural and functional homology to TDP-43. These observations provide further support that proper RNA metabolism is integrally involved in the development of

both ALS and FTD /173,174/. FUS/TLS is predominantly localized within the nucleus of healthy cells and shuttles between the nucleus and the cytoplasm, but in diseased neurons, FUS/TLS is excluded from the nucleus and accumulates in the cytoplasm, much like TDP-43. The neuropathologic features of neurodegeneration due to *TARDBP* and *FUS/TLS* mutations are inherently and fundamentally similar to one another /175/, suggesting that the disease pathogenesis might also be analogous in each case. It is unclear what effects pathogenic *FUS/TLS* mutations have upon the function or subcellular distribution of the protein and if these variables are important predictors of cell death and neurodegeneration.

Selective neuronal vulnerability in TDP-43 proteinopathies

TDP-43 is expressed ubiquitously, yet *TARDBP* mutations result in a neurodegenerative condition limited to motor neurons of the spinal cord, motor cortex, and selected populations of neurons in the frontal and temporal cortices. What cellular or molecular characteristics make these cells particularly susceptible to the toxic effects of mutant TDP-43? The specific factors that might contribute to neuronal selective vulnerability can be categorized into cell-autonomous and non-cell-autonomous traits. Intrinsic differences between susceptible populations of neurons within the cortex and spinal cord and resistant regions, such as the striatum or thalamus, may be responsible for the toxicity of TDP-43. For instance, motor neuron axon length or the apparent dependence of these cells on functional axon transport mechanisms /176/ might make anterior horn cells more susceptible. Alternatively, non-cell-autonomous variables, such as astrocyte or microglial inputs, could contribute to the vulnerability of cortical and spinal cord neurons /92,93/. In an *in vitro* model of mSOD-1-mediated MND, neuronal toxicity was traced to the secretion of a toxic factor from astrocytes expressing mSOD1 /177/. The precise contribution of astrocytes to TDP43-mediated disease is unclear, but these results suggest that inputs from adjacent astrocytes are essential for maintaining motor neuron health. Interestingly, while motor neurons are initially affected in transgenic rats expressing mutant TDP-43, more widespread pathology can be seen during late stages of disease, with degeneration noted throughout the cortex, hippocampus, and cerebellum /109/. Thus, while motor neurons appear to be preferentially susceptible to the toxic effects of mutant TDP-43, separate types of neurons and those residing in different regions of the nervous system may eventually be affected. Multisystem neuronal degeneration and protein deposition are

also characteristic pathologic features of ALS and FTD in humans, particularly in severe or late-stage illness /15-17,178/, suggesting that resistance to the toxicity of TDP-43 is relative.

While the majority of pathogenic *TARDBP* mutations have been associated with motor neuron disease, substantial heterogeneity exists in the clinical presentation of patients harboring *TARDBP* mutations, even within family members carrying identical mutations /80/. As described above, over 30 *TARDBP* mutations are associated with spontaneous or familial ALS in humans /59,63-78/. However, it is unclear if specific *TARDBP* mutations are associated with characteristic clinical phenotypes. Each of the mutations might lead to a unique set of downstream effects or preferential toxicity for limited neuronal populations, resulting in a particular distribution of clinical signs and symptoms. In support of this conclusion, transgenic mice expressing A315T mutant TDP-43 develop simultaneous motor and cortical neuron loss /108/, while transgenic rats expressing M337V mutant TDP-43 demonstrate early motor neuron degeneration and late cortical, limbic, and cerebellar involvement /109/. Furthermore, FTD is rarely found in association with *TARDBP* mutations, but two family members with the G295S mutation developed motor neuron disease with FTD /71/, and FTD without evidence of ALS has been detected in a single individual carrying the L263E mutation /72/. Mutant TDP-43 harboring these mutations may therefore be particularly toxic to cortical and motor neurons, through unknown mechanisms. Nevertheless, humans with identical *TARDBP* mutations exhibit different clinical phenotypes /80/, implying that one or more unidentified genetic or environmental factors significantly influence the onset, symptoms, and course of disease in *TARDBP* mutation carriers.

Conclusions

ALS and FTD are clinically distinct disorders that are linked through a common molecular pathology involving the cytoplasmic accumulation of TDP-43. As with other neurodegenerative conditions characterized by neuronal inclusions, one may wonder whether the protein constituents of these inclusions are directly involved in disease pathogenesis, or if they simply represent a downstream and non-specific phenomenon of neuronal dysfunction and death. A wealth of evidence, including anatomic, genetic, and biochemical studies involving *in vitro* and *in vivo* experimental systems, now supports a primary role for TDP-43 in the development of these diseases. Still, the precise pathways by which TDP-43 might be involved in disease pathogenesis are unclear,

and many questions remain. Do pathogenic *TARDBP* mutations induce neurotoxicity by incapacitating TDP-43 and preventing it from carrying out physiologic functions that are fundamentally essential for neuronal health? Or does mutant TDP-43 acquire new and potentially lethal properties that stimulate cell death independent of the normal function of the protein? Do mutations within *FUS/TLS* result in neurodegeneration through similar mechanisms? Why are neurons of the cortex and spinal cord preferentially affected by *TARDBP* or *FUS/TLS* mutations? Most importantly, answering these questions will further our understanding of an entire class of disorders characterized by TDP-43 deposition and promises to facilitate the development of more accurate disease models that recapitulate key features of TDP43-proteinopathies. Such models will be crucial if we are to design and evaluate therapies with the potential to prevent or reverse the signs, symptoms, and sequelae of ALS, FTD, and other devastating and presently incurable neurodegenerative conditions.

Bibliography

1. Cutler RG, Mattson MP. The adversities of aging. *Ageing Res Rev* 2006;5(3):221-38.
2. Ratnavalli E, Brayne C, Dawson K, Hodges JR. The prevalence of frontotemporal dementia. *Neurology* 2002;58(11):1615-21.
3. Diehl J, Kurz A. Frontotemporal dementia: patient characteristics, cognition, and behaviour. *Int J Geriatr Psychiatry* 2002;17(10):914-8.
4. Logroscino G, Traynor BJ, Hardiman O, Chio' A, Couratier P, Mitchell JD, Swingler RJ, Beghi E, EURALS. Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. *J Neurol Neurosurg Psychiatr* 2008;79(1):6-11.
5. Cima V, Logroscino G, D'Ascenzo C, Palmieri A, Volpe M, Briani C, Pegoraro E, Angelini C, Soraru G. Epidemiology of ALS in Padova district, Italy, from 1992 to 2005. *Eur J Neurol* 2009;16(8):920-4.
6. Bertram L, Tanzi RE. The genetic epidemiology of neurodegenerative disease. *J Clin Invest* 2005;115(6):1449-57.
7. Beghi E, Logroscino G, Chiò A, Hardiman O, Mitchell D, Swingler R, Traynor BJ, Consortium E. The epidemiology of ALS and the role of population-based registries. *Biochim Biophys Acta* 2006;1762(11-12):1150-7.
8. Shook SJ, Piro EP. Racing against the clock: recognizing, differentiating, diagnosing, and referring the amyotrophic lateral sclerosis patient. *Ann Neurol*. 2009;65 Suppl 1:S10-6.
9. Qureshi M, Schoenfeld DA, Paliwal Y, Shui A, Cudkowicz ME. The natural history of ALS is changing: improved survival. *Amyotroph Lateral Scler* 2009;10(5-6):324-31.

10. Bourke SC, Tomlinson M, Williams TL, Bullock RE, Shaw PJ, Gibson GJ. Effects of non-invasive ventilation on survival and quality of life in patients with amyotrophic lateral sclerosis: a randomised controlled trial. *Lancet Neurol* 2006;5(2):140-7.
11. Chiò A, Finocchiaro E, Meineri P, Bottacchi E, Schiffer D. Safety and factors related to survival after percutaneous endoscopic gastrostomy in ALS. ALS Percutaneous Endoscopic Gastrostomy Study Group. *Neurology* 1999;53(5):1123-5.
12. Dubinsky R, Chen J, Lai S-M. Trends in hospital utilization and outcome for patients with ALS: analysis of a large U.S. cohort. *Neurology* 2006;67(5):777-80.
13. Traynor BJ, Alexander M, Corr B, Frost E, Hardiman O. Effect of a multidisciplinary amyotrophic lateral sclerosis (ALS) clinic on ALS survival: a population based study, 1996-2000. *J Neurol Neurosurg Psychiatr* 2003;74(9):1258-61.
14. Borasio GD, Miller RG. Clinical characteristics and management of ALS. *Semin Neurol* 2001;21(2):155-66.
15. Geser F, Brandmeir NJ, Kwong LK, Martinez-Lage M, Elman L, McCluskey L, Xie SX, Lee VM-Y, Trojanowski JQ. Evidence of multisystem disorder in whole-brain map of pathological TDP-43 in amyotrophic lateral sclerosis. *Arch Neurol* 2008;65(5):636-41.
16. Geser F, Lee VM-Y, Trojanowski JQ. Amyotrophic lateral sclerosis and frontotemporal lobar degeneration: A spectrum of TDP-43 proteinopathies. *Neuropathology* Jan 25, 2010 [Epub ahead of print].
17. Geser F, Martinez-Lage M, Robinson J, Uryu K, Neumann M, Brandmeir NJ, Xie SX, Kwong LK, Elman L, McCluskey L, Clark CM, Malunda J, Miller BL, Zimmerman EA, Qian J, Van Deerlin V, Grossman M, Lee VM-Y, Trojanowski JQ. Clinical and pathological continuum of multisystem TDP-43 proteinopathies. *Arch Neurol* 2009;66(2):180-9.
18. Valdmanis PN, Rouleau GA. Genetics of familial amyotrophic lateral sclerosis. *Neurology* 2008;70(2):144-52.

19. Gros-Louis F, Gaspar C, Rouleau GA. Genetics of familial and sporadic amyotrophic lateral sclerosis. *Biochim Biophys Acta* 2006;1762(11-12):956-72.
20. Schymick JC, Talbot K, Traynor BJ. Genetics of sporadic amyotrophic lateral sclerosis. *Hum Mol Genet* 2007;16 Spec No. 2:R233-42.
21. Talbot K, Ansorge O. Recent advances in the genetics of amyotrophic lateral sclerosis and frontotemporal dementia: common pathways in neurodegenerative disease. *Hum Mol Genet* 2006;15 Spec No 2:R182-7.
22. Rabin BA, Griffin JW, Crain BJ, Scavina M, Chance PF, Cornblath DR. Autosomal dominant juvenile amyotrophic lateral sclerosis. *Brain* 1999;122 (Pt 8):1539-50.
23. Kato S. Amyotrophic lateral sclerosis models and human neuropathology: similarities and differences. *Acta Neuropathol* 2008;115(1):97-114.
24. Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. *Prog Neurobiol* 2008;85(1):94-134.
25. Brooks BR. Managing amyotrophic lateral sclerosis: slowing disease progression and improving patient quality of life. *Ann Neurol*. 2009;65 Suppl 1:S17-23.
26. Miller RG, Mitchell JD, Lyon M, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev* 2007(1):CD001447.
27. Practice advisory on the treatment of amyotrophic lateral sclerosis with riluzole: report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 1997;49(3):657-9.
28. Lomen-Hoerth C, Murphy J, Langmore S, Kramer JH, Olney RK, Miller B. Are amyotrophic lateral sclerosis patients cognitively normal? *Neurology* 2003;60(7):1094-7.
29. 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(4):530-4.

30. Graff-Radford NR, Woodruff BK. Frontotemporal dementia. *Semin Neurol* 2007;27(1):48-57.
31. Rankin KP, Baldwin E, Pace-Savitsky C, Kramer JH, Miller BL. Self awareness and personality change in dementia. *J Neurol Neurosurg Psychiatr* 2005;76(5):632-9.
32. Chang JL, Lomen-Hoerth C, Murphy J, Henry RG, Kramer JH, Miller BL, Gorno-Tempini ML. A voxel-based morphometry study of patterns of brain atrophy in ALS and ALS/FTLD. *Neurology* 2005;65(1):75-80.
33. Mori H, Yagishita A, Takeda T, Mizutani T. Symmetric temporal abnormalities on MR imaging in amyotrophic lateral sclerosis with dementia. *AJNR Am J Neuroradiol* 2007;28(8):1511-6.
34. Matsusue E, Sugihara S, Fujii S, Kinoshita T, Nakano T, Ohama E, Ogawa T. Cerebral cortical and white matter lesions in amyotrophic lateral sclerosis with dementia: correlation with MR and pathologic examinations. *AJNR Am J Neuroradiol* 2007;28(8):1505-10.
35. Ishihara K, Araki S, Ihori N, Shiota J-i, Kawamura M, Nakano I. An autopsy case of frontotemporal dementia with severe dysarthria and motor neuron disease showing numerous basophilic inclusions. *Neuropathology* 2006;26(5):447-54.
36. Shi J, Shaw CL, Du Plessis D, Richardson AMT, Bailey KL, Julien C, Stopford C, Thompson J, Varma A, Craufurd D, Tian J, Pickering-Brown S, Neary D, Snowden JS, Mann DMA. Histopathological changes underlying frontotemporal lobar degeneration with clinicopathological correlation. *Acta Neuropathol* 2005;110(5):501-12.
37. Mackenzie IRA, Bigio EH, Ince PG, Geser F, Neumann M, Cairns NJ, Kwong LK, Forman MS, Ravits J, Stewart H, Eisen A, McClusky L, Kretschmar HA, Monoranu CM, Highley JR, Kirby J, Siddique T, Shaw PJ, Lee VM-Y, Trojanowski JQ. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol.* 2007;61(5):427-34.
38. Mackenzie IRA, Feldman HH. Ubiquitin immunohistochemistry suggests classic motor neuron disease, motor neuron disease with dementia, and frontotemporal dementia of the motor neuron disease type represent a clinicopathologic spectrum. *J Neuropathol Exp Neurol* 2005;64(8):730-9.

39. Chow TW, Miller BL, Hayashi VN, Geschwind DH. Inheritance of frontotemporal dementia. *Arch Neurol* 1999;56(7):817-22.
40. Seelaar H, Kamphorst W, Rosso SM, Azmani A, Masdjedi R, de Koning I, Maat-Kievit JA, Anar B, Kaat LD, Breedveld GJ, Dooijes D, Rozemuller JM, Bronner IF, Rizzu P, van Swieten JC. Distinct genetic forms of frontotemporal dementia. *Neurology* 2008;71(16):1220-6.
41. Mackenzie IRA, Rademakers R. The molecular genetics and neuropathology of frontotemporal lobar degeneration: recent developments. *Neurogenetics* 2007;8(4):237-48.
42. Grossman M, Libon DJ, Forman MS, Massimo L, Wood E, Moore P, Anderson C, Farmer J, Chatterjee A, Clark CM, Coslett HB, Hurtig HI, Lee VM-Y, Trojanowski JQ. Distinct antemortem profiles in patients with pathologically defined frontotemporal dementia. *Arch Neurol* 2007;64(11):1601-9.
43. Rosen HJ, Gorno-Tempini ML, Goldman WP, Perry RJ, Schuff N, Weiner M, Feiwell R, Kramer JH, Miller BL. Patterns of brain atrophy in frontotemporal dementia and semantic dementia. *Neurology* 2002;58(2):198-208.
44. Woolley JD, Gorno-Tempini M-L, Seeley WW, Rankin K, Lee SS, Matthews BR, Miller BL. Binge eating is associated with right orbitofrontal-insular-striatal atrophy in frontotemporal dementia. *Neurology* 2007;69(14):1424-33.
45. Pereira JMS, Williams GB, Acosta-Cabronero J, Pengas G, Spillantini MG, Xuereb JH, Hodges JR, Nestor PJ. Atrophy patterns in histologic vs clinical groupings of frontotemporal lobar degeneration. *Neurology* 2009;72(19):1653-60.
46. Mackenzie IRA, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, Kovacs GG, Ghetti B, Halliday G, Holm IE, Ince PG, Kamphorst W, Revesz T, Rozemuller AJM, Kumar-Singh S, Akiyama H, Baborie A, Spina S, Dickson DW, Trojanowski JQ, Mann DMA. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. *Acta Neuropathol* 2010;119(1):1-4.

47. Neumann M, Rademakers R, Roeber S, Baker M, Kretzschmar HA, Mackenzie IRA. A new subtype of frontotemporal lobar degeneration with FUS pathology. *Brain* 2009;132(Pt 11):2922-31.
48. Neumann M. Molecular Neuropathology of TDP-43 Proteinopathies. *Int J Mol Sci* 2009;10(1):232-46.
49. Kwong LK, Neumann M, Sampathu DM, Lee VM-Y, Trojanowski JQ. TDP-43 proteinopathy: the neuropathology underlying major forms of sporadic and familial frontotemporal lobar degeneration and motor neuron disease. *Acta Neuropathol* 2007;114(1):63-70.
50. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM-Y. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006;314(5796):130-3.
51. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006;351(3):602-11.
52. Buratti E, Baralle FE. Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of CFTR exon 9. *J Biol Chem* 2001;276(39):36337-43.
53. Arai T, Hasegawa M, Nonaka T, Kametani F, Yamashita M, Hosokawa M, Niizato K, Tsuchiya K, Kobayashi Z, Ikeda K, Yoshida M, Onaya M, Fujishiro H, Akiyama H. Phosphorylated and cleaved TDP-43 in ALS, FTLN and other neurodegenerative disorders and in cellular models of TDP-43 proteinopathy. *Neuropathology* Jan 19, 2010 [Epub ahead of print].
54. Neumann M, Kwong LK, Lee EB, Kremmer E, Flatley A, Xu Y, Forman MS, Troost D, Kretzschmar HA, Trojanowski JQ, Lee VM-Y. Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. *Acta Neuropathol* 2009;117(2):137-49.

55. Inukai Y, Nonaka T, Arai T, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle FE, Akiyama H, Hisanaga S-i, Hasegawa M. Abnormal phosphorylation of Ser409/410 of TDP-43 in FTL-D-U and ALS. FEBS Letts. 2008;582(19):2899-904.
56. Nonaka T, Arai T, Buratti E, Baralle FE, Akiyama H, Hasegawa M. Phosphorylated and ubiquitinated TDP-43 pathological inclusions in ALS and FTL-D-U are recapitulated in SH-SY5Y cells. FEBS Letts. 2009;583(2):394-400.
57. Nonaka T, Kametani F, Arai T, Akiyama H, Hasegawa M. Truncation and pathogenic mutations facilitate the formation of intracellular aggregates of TDP-43. Hum Mol Genet 2009;18(18):3353-64.
58. Liu H-N, Sanelli T, Horne P, Piro EP, Strong MJ, Rogaeva E, Bilbao J, Zinman L, Robertson J. Lack of evidence of monomer/misfolded superoxide dismutase-1 in sporadic amyotrophic lateral sclerosis. Ann Neurol. 2009;66(1):75-80.
59. Van Deerlin VM, Leverenz JB, Bekris LM, Bird TD, Yuan W, Elman LB, Clay D, Wood EM, Chen-Plotkin AS, Martinez-Lage M, Steinbart E, McCluskey L, Grossman M, Neumann M, Wu I-L, Yang W-S, Kalb R, Galasko DR, Montine TJ, Trojanowski JQ, Lee VM-Y, Schellenberg GD, Yu C-E. TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. Lancet Neurol 2008;7(5):409-16.
60. Maekawa S, Leigh PN, King A, Jones E, Steele JC, Bodi I, Shaw CE, Hortobagyi T, Al-Sarraj S. 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 Jun 3, 2009 [Epub ahead of print].
61. Robertson J, Sanelli T, Xiao S, Yang W, Horne P, Hammond R, Piro EP, Strong MJ. Lack of TDP-43 abnormalities in mutant SOD1 transgenic mice shows disparity with ALS. Neurosci Lett 2007;420(2):128-32.
62. Gomes C, Escrevente C, Costa J. Mutant superoxide dismutase 1 overexpression in NSC-34 cells: effect of trehalose on aggregation, TDP-43 localization and levels of co-expressed glycoproteins. Neurosci Lett Apr 1, 2010 [Epub ahead of print].

63. Rutherford NJ, Zhang Y-J, Baker M, Gass JM, Finch NA, Xu Y-F, Stewart H, Kelley BJ, Kuntz K, Crook RJP, Sreedharan J, Vance C, Sorenson E, Lippa C, Bigio EH, Geschwind DH, Knopman DS, Mitsumoto H, Petersen RC, Cashman NR, Hutton M, Shaw CE, Boylan KB, Boeve B, Graff-Radford NR, Wszolek ZK, Caselli RJ, Dickson DW, Mackenzie IR, Petrucelli L, Rademakers R. Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. *PLoS Genet* 2008;4(9):e1000193.
64. Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, Ackerley S, Durnall JC, Williams KL, Buratti E, Baralle F, de Belleruche J, Mitchell JD, Leigh PN, Al-Chalabi A, Miller CC, Nicholson G, Shaw CE. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 2008;319(5870):1668-72.
65. Kühnlein P, Sperfeld A-D, Vanmassenhove B, Van Deerlin V, Lee VM-Y, Trojanowski JQ, Kretzschmar HA, Ludolph AC, Neumann M. Two German kindreds with familial amyotrophic lateral sclerosis due to TARDBP mutations. *Arch Neurol* 2008;65(9):1185-9.
66. Kabashi E, Valdmanis PN, Dion P, Spiegelman D, McConkey BJ, Vande Velde C, Bouchard J-P, Lacomblez L, Pochigaeva K, Salachas F, Pradat P-F, Camu W, Meininger V, Dupre N, Rouleau GA. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet* 2008;40(5):572-4.
67. Luquin N, Yu B, Saunderson RB, Trent RJ, Pamphlett R. Genetic variants in the promoter of TARDBP in sporadic amyotrophic lateral sclerosis. *Neuromuscul Disord* 2009;19(10):696-700.
68. Gitcho MA, Baloh RH, Chakraverty S, Mayo K, Norton JB, Levitch D, Hatanpaa KJ, White CL, Bigio EH, Caselli R, Baker M, Al-Lozi MT, Morris JC, Pestronk A, Rademakers R, Goate AM, Cairns NJ. TDP-43 A315T mutation in familial motor neuron disease. *Ann Neurol*. 2008;63(4):535-8.
69. Gitcho MA, Bigio EH, Mishra M, Johnson N, Weintraub S, Mesulam M, Rademakers R, Chakraverty S, Cruchaga C, Morris JC, Goate AM, Cairns NJ. TARDBP 3'-UTR variant in autopsy-confirmed frontotemporal lobar degeneration with TDP-43 proteinopathy. *Acta Neuropathol* 2009;118(5):633-45.
70. Bäumer D, Parkinson N, Talbot K. TARDBP in amyotrophic lateral sclerosis: identification of a novel variant but absence of copy number variation. *J Neurol Neurosurg Psychiatr* 2009;80(11):1283-5.

71. Benajiba L, Le Ber I, Camuzat A, Lacoste M, Thomas-Anterion C, Couratier P, Legallic S, Salachas F, Hannequin D, Decousus M, Lacomblez L, Guedj E, Golfier V, Camu W, Dubois B, Campion D, Meininger V, Brice A, Disease FCaGRNoFLDFLDwM. TARDBP mutations in motoneuron disease with frontotemporal lobar degeneration. *Ann Neurol*. 2009;65(4):470-3.
72. Kovacs GG, Murrell JR, Horvath S, Haraszti L, Majtenyi K, Molnar MJ, Budka H, Ghetti B, Spina S. TARDBP variation associated with frontotemporal dementia, supranuclear gaze palsy, and chorea. *Mov Disord* 2009;24(12):1843-7.
73. Corrado L, Ratti A, Gellera C, Buratti E, Castellotti B, Carlomagno Y, Ticozzi N, Mazzini L, Testa L, Taroni F, Baralle FE, Silani V, D'Alfonso S. High frequency of TARDBP gene mutations in Italian patients with amyotrophic lateral sclerosis. *Hum Mutat* 2009;30(4):688-94.
74. Del Bo R, Ghezzi S, Corti S, Pandolfo M, Ranieri M, Santoro D, Ghione I, Prella A, Orsetti V, Mancuso M, Soraru G, Briani C, Angelini C, Siciliano G, Bresolin N, Comi GP. TARDBP (TDP-43) sequence analysis in patients with familial and sporadic ALS: identification of two novel mutations. *Eur J Neurol* 2009;16(6):727-32.
75. Lemmens R, Race V, Hersmus N, Matthijs G, Van Den Bosch L, Van Damme P, Dubois B, Boonen S, Goris A, Robberecht W. TDP-43 M311V mutation in familial amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatr* 2009;80(3):354-5.
76. Yokoseki A, Shiga A, Tan C-F, Tagawa A, Kaneko H, Koyama A, Eguchi H, Tsujino A, Ikeuchi T, Kakita A, Okamoto K, Nishizawa M, Takahashi H, Onodera O. TDP-43 mutation in familial amyotrophic lateral sclerosis. *Ann Neurol*. 2008;63(4):538-42.
77. Kamada M, Maruyama H, Tanaka E, Morino H, Wate R, Ito H, Kusaka H, Kawano Y, Miki T, Nodera H, Izumi Y, Kaji R, Kawakami H. Screening for TARDBP mutations in Japanese familial amyotrophic lateral sclerosis. *J Neurol Sci* 2009;284(1-2):69-71.
78. Daoud H, Valdmanis PN, Kabashi E, Dion P, Dupré N, Camu W, Meininger V, Rouleau GA. Contribution of TARDBP mutations to sporadic amyotrophic lateral sclerosis. *J Med Genet* 2009;46(2):112-4.

79. Ticozzi N, Leclerc AL, van Blitterswijk M, Keagle P, McKenna-Yasek DM, Sapp PC, Silani V, Wills A-M, Brown RH, Landers JE. Mutational analysis of TARDBP in neurodegenerative diseases. *Neurobiol Aging* Dec 21, 2009 [Epub ahead of print].
80. Kirby J, Goodall EF, Smith W, Highley JR, Masanzu R, Hartley JA, Hibberd R, Hollinger HC, Wharton SB, Morrison KE, Ince PG, McDermott CJ, Shaw PJ. Broad clinical phenotypes associated with TAR-DNA binding protein (TARDBP) mutations in amyotrophic lateral sclerosis. *Neurogenetics* 2009;11(2):217-25.
81. Cairns NJ, Neumann M, Bigio EH, Holm IE, Troost D, Hatanpaa KJ, Foong C, White CL, Schneider JA, Kretzschmar HA, Carter D, Taylor-Reinwald L, Paulsmeyer K, Strider J, Gitcho M, Goate AM, Morris JC, Mishra M, Kwong LK, Stieber A, Xu Y, Forman MS, Trojanowski JQ, Lee VM-Y, Mackenzie IRA. TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol* 2007;171(1):227-40.
82. Le Ber I, Camuzat A, Berger E, Hannequin D, Laquerrière A, Golfier V, Seilhean D, Viennet G, Couratier P, Verpillat P, Heath S, Camu W, Martinaud O, Lacomblez L, Vercelletto M, Salachas F, Sellal F, Didic M, Thomas-Anterion C, Puel M, Michel B-F, Besse C, Duyckaerts C, Meininger V, Campion D, Dubois B, Brice A, FTD/FTD-MND FRNo. Chromosome 9p-linked families with frontotemporal dementia associated with motor neuron disease. *Neurology* 2009;72(19):1669-76.
83. Luty AA, Kwok JBJ, Thompson EM, Blumbergs P, Brooks WS, Loy CT, Dobson-Stone C, Panegyres PK, Hecker J, Nicholson GA, Halliday GM, Schofield PR. Pedigree with frontotemporal lobar degeneration--motor neuron disease and Tar DNA binding protein-43 positive neuropathology: genetic linkage to chromosome 9. *BMC Neurol* 2008;8:32.
84. Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, Snowden J, Adamson J, Sadovnick AD, Rollinson S, Cannon A, Dwosh E, Neary D, Melquist S, Richardson A, Dickson D, Berger Z, Eriksen J, Robinson T, Zehr C, Dickey CA, Crook R, McGowan E, Mann D, Boeve B, Feldman H, Hutton M. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 2006;442(7105):916-9.

85. Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, Rademakers R, Vandenberghe R, Dermaut B, Martin J-J, van Duijn C, Peeters K, Sciot R, Santens P, De Pooter T, Mattheijssens M, Van den Broeck M, Cuijt I, Vennekens KI, De Deyn PP, Kumar-Singh S, Van Broeckhoven C. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 2006;442(7105):920-4.
86. Shankaran SS, Capell A, Hruscha AT, Fellerer K, Neumann M, Schmid B, Haass C. Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive inclusions reduce progranulin production and secretion. *J Biol Chem* 2008;283(3):1744-53.
87. van Swieten JC, Heutink P. Mutations in progranulin (GRN) within the spectrum of clinical and pathological phenotypes of frontotemporal dementia. *Lancet Neurol* 2008;7(10):965-74.
88. Kimonis VE, Fulchiero E, Vesa J, Watts G. VCP disease associated with myopathy, Paget disease of bone and frontotemporal dementia: review of a unique disorder. *Biochim Biophys Acta* 2008;1782(12):744-8.
89. Watts GDJ, Wymer J, Kovach MJ, Mehta SG, Mumm S, Darvish D, Pestronk A, Whyte MP, Kimonis VE. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat Genet* 2004;36(4):377-81.
90. Mackenzie IRA, Rademakers R. The role of transactive response DNA-binding protein-43 in amyotrophic lateral sclerosis and frontotemporal dementia. *Curr Opin Neurol* 2008;21(6):693-700.
91. Schymick JC, Yang Y, Andersen PM, Vonsattel JP, Greenway M, Momeni P, Elder J, Chiò A, Restagno G, Robberecht W, Dahlberg C, Mukherjee O, Goate A, Graff-Radford N, Caselli RJ, Hutton M, Gass J, Cannon A, Rademakers R, Singleton AB, Hardiman O, Rothstein J, Hardy J, Traynor BJ. Progranulin mutations and amyotrophic lateral sclerosis or amyotrophic lateral sclerosis-frontotemporal dementia phenotypes. *J Neurol Neurosurg Psychiatr* 2007;78(7):754-6.
92. Boillée S, Vande Velde C, Cleveland DW. ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* 2006;52(1):39-59.

93. Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat Rev Neurosci* 2001;2(11):806-19.
94. Ilieva H, Polymenidou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J Cell Biol* 2009;187(6):761-72.
95. Seeley WW. Selective functional, regional, and neuronal vulnerability in frontotemporal dementia. *Curr Opin Neurol* 2008;21(6):701-7.
96. Agorogiannis EI, Agorogiannis GI, Papadimitriou A, Hadjigeorgiou GM. Protein misfolding in neurodegenerative diseases. *Neuropath App Neuro* 2004;30(3):215-24.
97. Soto C, Estrada LD. Protein misfolding and neurodegeneration. *Arch Neurol* 2008;65(2):184-9.
98. Zhang Q, Zhang X, Sun A. Truncated tau at D421 is associated with neurodegeneration and tangle formation in the brain of Alzheimer transgenic models. *Acta Neuropathol* 2009;117(6):687-97.
99. Adams SJ, Crook RJP, Deture M, Randle SJ, Innes AE, Yu XZ, Lin W-L, Dugger BN, McBride M, Hutton M, Dickson DW, McGowan E. Overexpression of wild-type murine tau results in progressive tauopathy and neurodegeneration. *Am J Pathol* 2009;175(4):1598-609.
100. Tagliavini F, Lievens PM, Tranchant C, Warter JM, Mohr M, Giaccone G, Perini F, Rossi G, Salmona M, Piccardo P, Ghetti B, Beavis RC, Bugiani O, Frangione B, Prelli F. A 7-kDa prion protein (PrP) fragment, an integral component of the PrP region required for infectivity, is the major amyloid protein in Gerstmann-Sträussler-Scheinker disease A117V. *J Biol Chem* 2001;276(8):6009-15.
101. Johnson BS, McCaffery JM, Lindquist S, Gitler AD. A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. *Proc Natl Acad Sci USA* 2008;105(17):6439-44.
102. Zhang Y-J, Xu Y-F, Cook C, Gendron TF, Roettges P, Link CD, Lin W-L, Tong J, Castanedes-Casey M, Ash P, Gass J, Rangachari V, Buratti E, Baralle F, Golde TE, Dickson DW, Petrucelli L. Aberrant cleavage of TDP-43 enhances aggregation and cellular toxicity. *Proc Natl Acad Sci USA* 2009;106(18):7607-12.

103. Chen AK-H, Lin RY-Y, Hsieh EZ-J, Tu P-H, Chen RP-Y, Liao T-Y, Chen W, Wang C-H, Huang JJ-T. Induction of amyloid fibrils by the C-terminal fragments of TDP-43 in amyotrophic lateral sclerosis. *J Am Chem Soc* 2010;132(4):1186-7.
104. Barmada SJ, Skibinski G, Korb E, Rao EJ, Wu JY, Finkbeiner S. Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral sclerosis. *J Neurosci* 2010;30(2):639-49.
105. Kabashi E, Lin L, Tradewell ML, Dion PA, Bercier V, Bourguoin P, Rochefort D, Bel Hadj S, Durham HD, Vande Velde C, Rouleau GA, Drapeau P. Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits in vivo. *Hum Mol Genet* 2010;19(4):671-83.
106. Tatom JB, Wang DB, Dayton RD, Skalli O, Hutton ML, Dickson DW, Klein RL. Mimicking aspects of frontotemporal lobar degeneration and Lou Gehrig's disease in rats via TDP-43 overexpression. *Mol Ther* 2009;17(4):607-13.
107. Wils H, Kleinberger G, Janssens J, Pereson S, Joris G, Cuijt I, Smits V, Ceuterick-de Groote C, Van Broeckhoven C, Kumar-Singh S. TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. *Proc Natl Acad Sci U S A* 2010;107(8):3858-63.
108. Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH. TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. *Proc Natl Acad Sci USA* 2009;106(44):18809-14.
109. Zhou H, Huang C, Chen H, Wang D, Landel CP, Xia PY, Bowser R, Liu YJ, Xia XG. Transgenic rat model of neurodegeneration caused by mutation in the TDP gene. *PLoS Genet* 2010;6(3):e1000887.
110. Ou SH, Wu F, Harrich D, García-Martínez LF, Gaynor RB. Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. *J Virol* 1995;69(6):3584-96.
111. Stover CM, Lynch NJ, Hanson SJ, Windbichler M, Gregory SG, Schwaeble WJ. Organization of the MASP2 locus and its expression profile in mouse and rat. *Mamm Genome* 2004;15(11):887-900.

112. Ayala YM, Pantano S, D'Ambrogio A, Buratti E, Brindisi A, Marchetti C, Romano M, Baralle FE. Human, *Drosophila*, and *C.elegans* TDP43: nucleic acid binding properties and splicing regulatory function. *J Mol Biol* 2005;348(3):575-88.
113. Wang H-Y, Wang I-F, Bose J, Shen C-KJ. Structural diversity and functional implications of the eukaryotic TDP gene family. *Genomics* 2004;83(1):130-9.
114. Pesiridis GS, Lee VM-Y, Trojanowski JQ. Mutations in TDP-43 link glycine-rich domain functions to amyotrophic lateral sclerosis. *Hum Mol Genet* 2009;18(R2):R156-62.
115. Acharya KK, Govind CK, Shore AN, Stoler MH, Reddi PP. cis-requirement for the maintenance of round spermatid-specific transcription. *Dev Biol* 2006;295(2):781-90.
116. Buratti E, Dörk T, Zuccato E, Pagani F, Romano M, Baralle FE. Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. *EMBO J* 2001;20(7):1774-84.
117. Mercado PA, Ayala YM, Romano M, Buratti E, Baralle FE. Depletion of TDP 43 overrides the need for exonic and intronic splicing enhancers in the human apoA-II gene. *Nucleic Acids Res* 2005;33(18):6000-10.
118. Bose JK, Wang I-F, Hung L, Tarn W-Y, Shen C-KJ. TDP-43 overexpression enhances exon 7 inclusion during the survival of motor neuron pre-mRNA splicing. *J Biol Chem* 2008;283(43):28852-9.
119. Wang I-F, Reddy NM, Shen C-KJ. Higher order arrangement of the eukaryotic nuclear bodies. *Proc Natl Acad Sci USA* 2002;99(21):13583-8.
120. Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995;80(1):155-65.
121. Fukuda T, Yamagata K, Fujiyama S, Matsumoto T, Koshida I, Yoshimura K, Mihara M, Naitou M, Endoh H, Nakamura T, Akimoto C, Yamamoto Y, Katagiri T, Foulds C, Takezawa S, Kitagawa H, Takeyama K-i, O'Malley BW, Kato S. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat Cell Biol* 2007;9(5):604-11.

122. Strong MJ, Volkening K, Hammond R, Yang W, Strong W, Leystra-Lantz C, Shoesmith C. TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. *Mol Cell Neurosci* 2007;35(2):320-7.
123. Volkening K, Leystra-Lantz C, Yang W, Jaffee H, Strong MJ. Tar DNA binding protein of 43 kDa (TDP-43), 14-3-3 proteins and copper/zinc superoxide dismutase (SOD1) interact to modulate NFL mRNA stability. Implications for altered RNA processing in amyotrophic lateral sclerosis (ALS). *Brain Res* 2009;1305:168-82.
124. Wong NK, He BP, Strong MJ. Characterization of neuronal intermediate filament protein expression in cervical spinal motor neurons in sporadic amyotrophic lateral sclerosis (ALS). *J Neuropathol Exp Neurol* 2000;59(11):972-82.
125. Xu Z, Cork LC, Griffin JW, Cleveland DW. Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. *Cell* 1993;73(1):23-33.
126. Julien JP, Cote F, Collard JF. Mice overexpressing the human neurofilament heavy gene as a model of ALS. *Neurobiol Aging* 1995;16(3):487-90; discussion 90-2.
127. Strong MJ. The evidence for altered RNA metabolism in amyotrophic lateral sclerosis (ALS). *J Neurol Sci* 2010;288(1-2):1-12.
128. Wang I-F, Wu L-S, Chang H-Y, Shen C-KJ. TDP-43, the signature protein of FTL-D-U, is a neuronal activity-responsive factor. *J Neurochem* 2008;105(3):797-806.
129. Ayala YM, Zago P, D'Ambrogio A, Xu Y-F, Petrucelli L, Buratti E, Baralle FE. Structural determinants of the cellular localization and shuttling of TDP-43. *J Cell Sci* 2008;121(Pt 22):3778-85.
130. Winton MJ, Igaz LM, Wong MM, Kwong LK, Trojanowski JQ, Lee VM-Y. Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. *J Biol Chem* 2008;283(19):13302-9.

131. Elvira G, Wasiak S, Blandford V, Tong X-K, Serrano A, Fan X, del Rayo Sánchez-Carbente M, Servant F, Bell AW, Boismenu D, Lacaille J-C, McPherson PS, DesGroseillers L, Sossin WS. Characterization of an RNA granule from developing brain. *Mol Cell Proteomics* 2006;5(4):635-51.
132. Nishimoto Y, Ito D, Yagi T, Nihei Y, Tsunoda Y, Suzuki N. Characterization of alternative isoforms and inclusion body of the TAR DNA-binding protein-43. *J Biol Chem* 2010;285(1):608-19.
133. Moisse K, Volkening K, Leystra-Lantz C, Welch I, Hill T, Strong MJ. Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy: implications for TDP-43 in the physiological response to neuronal injury. *Brain Res* 2009;1249:202-11.
134. Colombrita C, Zennaro E, Fallini C, Weber M, Sommacal A, Buratti E, Silani V, Ratti A. TDP-43 is recruited to stress granules in conditions of oxidative insult. *J Neurochem* 2009;111(4):1051-61.
135. Freibaum BD, Chitta RK, High AA, Taylor JP. Global Analysis of TDP-43 Interacting Proteins Reveals Strong Association with RNA Splicing and Translation Machinery. *J Proteome Res* 2010;9(2):1104-20.
136. Lin AC, Holt CE. Function and regulation of local axonal translation. *Curr Opin Neurobiol* 2008;18(1):60-8.
137. Sato T, Takeuchi S, Saito A, Ding W, Bamba H, Matsuura H, Hisa Y, Tooyama I, Urushitani M. Axonal ligation induces transient redistribution of TDP-43 in brainstem motor neurons. *Neuroscience* 2009;164(4):1565-78.
138. Moisse K, Mephram J, Volkening K, Welch I, Hill T, Strong MJ. Cytosolic TDP-43 expression following axotomy is associated with caspase 3 activation in NFL^{-/-} mice: support for a role for TDP-43 in the physiological response to neuronal injury. *Brain Res* 2009;1296:176-86.
139. Nakamura M, Ito H, Wate R, Nakano S, Hirano A, Kusaka H. Phosphorylated Smad2/3 immunoreactivity in sporadic and familial amyotrophic lateral sclerosis and its mouse model. *Acta Neuropathol* 2008;115(3):327-34.

140. Tesseur I, Zou K, Esposito L, Bard F, Berber E, Can JV, Lin AH, Crews L, Tremblay P, Mathews P, Mucke L, Masliah E, Wyss-Coray T. Deficiency in neuronal TGF-beta signaling promotes neurodegeneration and Alzheimer's pathology. *J Clin Invest* 2006;116(11):3060-9.
141. Wu LS, Cheng WC, Hou SC, Yan YT, Jiang ST, Shen CK. TDP-43, a neuro-pathosignature factor, is essential for early mouse embryogenesis. *Genesis* 2010;48(1):56-62.
142. Sephton CF, Good SK, Atkin S, Dewey CM, Mayer P, 3rd, Herz J, Yu G. TDP-43 is a developmentally regulated protein essential for early embryonic development. *J Biol Chem* 2009;285(9):6826-34.
143. Fiesel FC, Voigt A, Weber SS, Van den Haute C, Waldenmaier A, Görner K, Walter M, Anderson ML, Kern JV, Rasse TM, Schmidt T, Springer W, Kirchner R, Bonin M, Neumann M, Baekelandt V, Alunni-Fabroni M, Schulz JB, Kahle PJ. Knockdown of transactive response DNA-binding protein (TDP-43) downregulates histone deacetylase 6. *EMBO J* 2010;29(1):209-21.
144. Feiguin F, Godena VK, Romano G, D'Ambrogio A, Klima R, Baralle FE. Depletion of TDP-43 affects *Drosophila* motoneurons terminal synapsis and locomotive behavior. *FEBS Letts.* 2009;583(10):1586-92.
145. Li Y, Ray P, Rao EJ, Shi C, Guo W, Chen X, Woodruff EA, 3rd, Fushimi K, Wu JY. A *Drosophila* model for TDP-43 proteinopathy. *Proc Natl Acad Sci U S A* 2010;107(7):3169-74.
146. Lu Y, Ferris J, Gao F-B. Frontotemporal dementia and amyotrophic lateral sclerosis-associated disease protein TDP-43 promotes dendritic branching. *Mol Brain* 2009;2(1):30.
147. Yu CE, Bird TD, Bekris LM, Montine TJ, Leverenz JB, Steinbart E, Galloway NM, Feldman H, Woltjer R, Miller CA, Wood EM, Grossman M, McCluskey L, Clark CM, Neumann M, Danek A, Galasko DR, Arnold SE, Chen-Plotkin A, Karydas A, Miller BL, Trojanowski JQ, Lee VM, Schellenberg GD, Van Deerlin VM. The spectrum of mutations in progranulin: a collaborative study screening 545 cases of neurodegeneration. *Arch Neurol* 2010;67(2):161-70.
148. Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, Brandner S, Brun A, Rossor MN, Gade A, Johannsen P, Sørensen SA, Gydesen

- S, Fisher EMC, Collinge J. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet* 2005;37(8):806-8.
149. Lee J-A, Gao F-B. Inhibition of autophagy induction delays neuronal cell loss caused by dysfunctional ESCRT-III in frontotemporal dementia. *J Neurosci* 2009;29(26):8506-11.
150. Filimonenko M, Stuffers S, Raiborg C, Yamamoto A, Malerød L, Fisher EMC, Isaacs A, Brech A, Stenmark H, Simonsen A. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. *J Cell Biol* 2007;179(3):485-500.
151. Ju J-S, Fuentealba RA, Miller SE, Jackson E, Piwnicka-Worms D, Baloh RH, Weihl CC. Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J Cell Biol* 2009;187(6):875-88.
152. Caccamo A, Majumder S, Deng JJ, Bai Y, Thornton FB, Oddo S. Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability. *J Biol Chem* 2009;284(40):27416-24.
153. Shiina Y, Arima K, Tabunoki H, Satoh JI. TDP-43 Dimerizes in Human Cells in Culture. *Cell Mol Neurobiol* Dec 31, 2009 [Epub ahead of print].
154. Zoghbi HY, Orr HT. Pathogenic mechanisms of a polyglutamine-mediated neurodegenerative disease, spinocerebellar ataxia type 1. *J Biol Chem* 2009;284(12):7425-9.
155. Fioriti L, Quaglio E, Massignan T, Colombo L, Stewart RS, Salmona M, Harris DA, Forloni G, Chiesa R. The neurotoxicity of prion protein (PrP) peptide 106-126 is independent of the expression level of PrP and is not mediated by abnormal PrP species. *Mol Cell Neurosci* 2005;28(1):165-76.
156. La Spada AR, Paulson HL, Fischbeck KH. Trinucleotide repeat expansion in neurological disease. *Ann Neurol*. 1994;36(6):814-22.
157. Deng H-X, Han-Xiang D, Jiang H, Hujun J, Fu R, Ronggen F, Zhai H, Hong Z, Shi Y, Yong S, Liu E, Erdong L, Hirano M, Makito H, Dal Canto MC, Mauro CDC, Siddique T, Teepu S. Molecular dissection of ALS-

associated toxicity of SOD1 in transgenic mice using an exon-fusion approach. *Hum Mol Genet* 2008;17(15):2310-9.

158. Govoni S, Bergamaschi S, Gasparini L, Quaglia C, Racchi M, Cattaneo E, Binetti G, Bianchetti A, Giovetti F, Battaini F, Trabuechi M. Fibroblasts of patients affected by Down's syndrome oversecrete amyloid precursor protein and are hyporesponsive to protein kinase C stimulation. *Neurology* 1996;47(4):1069-75.

159. Cheng SV, Nadeau JH, Tanzi RE, Watkins PC, Jagadesh J, Taylor BA, Haines JL, Sacchi N, Gusella JF. Comparative mapping of DNA markers from the familial Alzheimer disease and Down syndrome regions of human chromosome 21 to mouse chromosomes 16 and 17. *Proc Natl Acad Sci USA* 1988;85(16):6032-6.

160. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 2003;302(5646):841.

161. Hanson KA, Kim SH, Wassarman DA, Tibbetts RS. Ubiquitin modifies TDP-43 toxicity in a *Drosophila* model of amyotrophic lateral sclerosis (ALS). *J Biol Chem* 2010;285(15):11068-72.

162. Suzuki M, Mikami H, Watanabe T, Yamano T, Yamazaki T, Nomura M, Yasui K, Ishikawa H, Ono S. Increased expression of TDP-43 in the skin of amyotrophic lateral sclerosis. *Acta Neurol Scand* Feb 17, 2010 [Epub ahead of print].

163. Ranum LP, Cooper TA. RNA-mediated neuromuscular disorders. *Annu Rev Neurosci* 2006;29:259-77.

164. Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. *Neuron* 2003;40(2):427-46.

165. Kim SH, Shi Y, Hanson KA, Williams LM, Sakasai R, Bowler MJ, Tibbetts RS. Potentiation of Amyotrophic Lateral Sclerosis (ALS)-associated TDP-43 Aggregation by the Proteasome-targeting Factor, Ubiquitin 1. *J Biol Chem* 2009;284(12):8083-92.

166. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 2004;431(7010):805-10.
167. Ko L-W, Ko H-HC, Lin W-L, Kulathingal JG, Yen S-HC. Aggregates assembled from overexpression of wild-type alpha-synuclein are not toxic to human neuronal cells. *J Neuropathol Exp Neurol* 2008;67(11):1084-96.
168. Lesné S, Kotilinek L, Ashe KH. Plaque-bearing mice with reduced levels of oligomeric amyloid-beta assemblies have intact memory function. *Neuroscience* 2008;151(3):745-9.
169. Mitra S, Tsvetkov AS, Finkbeiner S. Single neuron ubiquitin-proteasome dynamics accompanying inclusion body formation in huntington disease. *J Biol Chem* 2009;284(7):4398-403.
170. Berlau DJ, Corrada MM, Head E, Kawas CH. APOE epsilon2 is associated with intact cognition but increased Alzheimer pathology in the oldest old. *Neurology* 2009;72(9):829-34.
171. Johnson BS, Snead D, Lee JJ, Mccaffery JM, Shorter J, Gitler AD. TDP-43 Is Intrinsically Aggregation-prone, and Amyotrophic Lateral Sclerosis-linked Mutations Accelerate Aggregation and Increase Toxicity. *J Biol Chem* 2009;284(30):20329-39.
172. Pamphlett R, Kum Jew S. TDP-43 inclusions do not protect motor neurons from sporadic ALS. *Acta Neuropathol* 2008;116(2):221-2.
173. Kwiatkowski TJ, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, Valdmanis P, Rouleau GA, Hosler BA, Cortelli P, de Jong PJ, Yoshinaga Y, Haines JL, Pericak-Vance MA, Yan J, Ticozzi N, Siddique T, Mckenna-Yasek D, Sapp PC, Horvitz HR, Landers JE, Brown RH. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 2009;323(5918):1205-8.
174. Vance C, Rogelj B, Hortobágyi T, De Vos KJ, Nishimura AL, Sreedharan J, Hu X, Smith B, Ruddy D, Wright P, Ganesalingam J, Williams KL, Tripathi V, Al-Saraj S, Al-Chalabi A, Leigh PN, Blair IP, Nicholson G,

de Belleruche J, Gallo J-M, Miller CC, Shaw CE. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 2009;323(5918):1208-11.

175. Blair IP, Williams KL, Warraich ST, Durnall JC, Thoeng AD, Manavis J, Blumbergs PC, Vucic S, Kiernan MC, Nicholson GA. FUS mutations in amyotrophic lateral sclerosis: clinical, pathological, neurophysiological and genetic analysis. *J Neurol Neurosurg Psychiatr* Dec 3, 2009 [Epub ahead of print].

176. Holzbaur ELF. Motor neurons rely on motor proteins. *Trends Cell Biol* 2004;14(5):233-40.

177. Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci* 2007;10(5):615-22.

178. van der Graaff MM, de Jong JMBV, Baas F, de Visser M. Upper motor neuron and extra-motor neuron involvement in amyotrophic lateral sclerosis: a clinical and brain imaging review. *Neuromuscul Disord* 2009;19(1):53-8.

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Table: Pathogenic *TARDBP* mutations and their associated features.

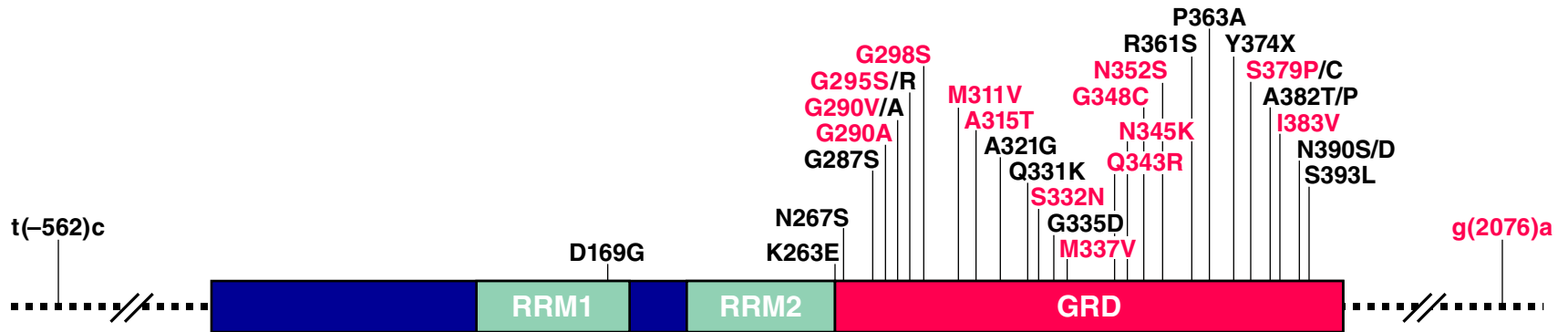
Domain	Mutation	Inheritance	Clinical presentation	Reference
5'-UTR	t(-562)c	Spont.	ALS	67
RRM1	D169G	Spont.	ALS	66
RRM2	K263E	Spont.	FTD, chorea, gaze palsy	72
GRD	N267S	Spont.	ALS	73
GRD	G287S	Spont.	ALS	66, 73
GRD	G290A	AD	ALS	59
GRD	G294A	Spont.	ALS	64
GRD	G294V	AD/Spont.	ALS	73, 74
GRD	G295R	Spont.	ALS	73
GRD	G295S	AD/Spont.	FTD-MND, ALS	71, 73, 74
GRD	G298S	AD	ALS	59
GRD	M311V	AD	ALS	75
GRD	A315T	AD	ALS	66
GRD	A321G	Spont.	ALS	70
GRD	Q331K	Spont.	ALS	64
GRD	S332N	AD	ALS	73
GRD	G335D	Spont.	ALS	73
GRD	M337V	AD	ALS	63, 64, 73
GRD	Q343R	AD	ALS	76
GRD	N345K	AD	ALS	63
GRD	G348C	AD/Spont.	ALS	65, 66, 74, 78
GRD	N352S	AD	ALS	65, 77
GRD	R361S	Spont.	ALS	66
GRD	P363A	Spont.	ALS	78
GRD	Y374X	Spont.	ALS	78
GRD	S379C	Spont.	ALS	73
GRD	S379P	AD	ALS	73
GRD	A382P	Spont.	ALS	78
GRD	A382T	AD/Spont.	ALS	66, 73, 74
GRD	I383V	AD	ALS	63
GRD	N390D	Spont.	ALS	66
GRD	N390S	Spont.	ALS	66
GRD	S393L	Spont.	ALS	73
3'-UTR	g(2076)a	AD	FTD, ALS	69

UTR: untranslated region; GRD: glycine-rich domain; RRM: RNA-recognition motif; AD: autosomal dominant; Spont: spontaneous; ALS: amyotrophic lateral sclerosis; FTD: frontotemporal dementia; FTD-MND: frontotemporal dementia with motor neuron disease.

Figure legends

Figure 1: Pathogenic *TARDBP* mutations cluster within the glycine-rich domain of the protein. A domain structure map of TDP-43 shows the RNA-recognition motifs 1 and 2 (RRM1 and RRM2, respectively) in light blue and the glycine-rich domain (GRD) in red. Black lines denote the approximate location of each mutation. Mutations in red represent those that have been associated with autosomal dominant (AD) disease; those in black occurred spontaneously (Spont.). The t(-562)c mutation (far left) occurs in the 5'-untranslated region (UTR) of the locus, at a position 562 base-pairs upstream of the transcription initiation site in exon 1. The g(2076)a mutation (far right) is located 2076 base-pairs downstream of transcriptional start site, in the 3'-UTR. Each of the individual mutations, the domains in which they are located, their inheritance patterns and associated clinical features are listed in the Table.

Figure 2: Several essential functions of TDP-43 in the nucleus and cytoplasm might contribute to disease pathogenesis. A motor neuron (center) is depicted, although TDP43-specific processes likely affect cortical as well as spinal neurons. Nuclear functions attributed to TDP-43 are listed above the neuron, and cytoplasmic functions appear below. The left-hand column of each table describes the proposed physiologic functions of TDP-43. The possible ways in which these functions might be perturbed in TDP43-proteinopathies are shown in the right-hand column of each of the tables.



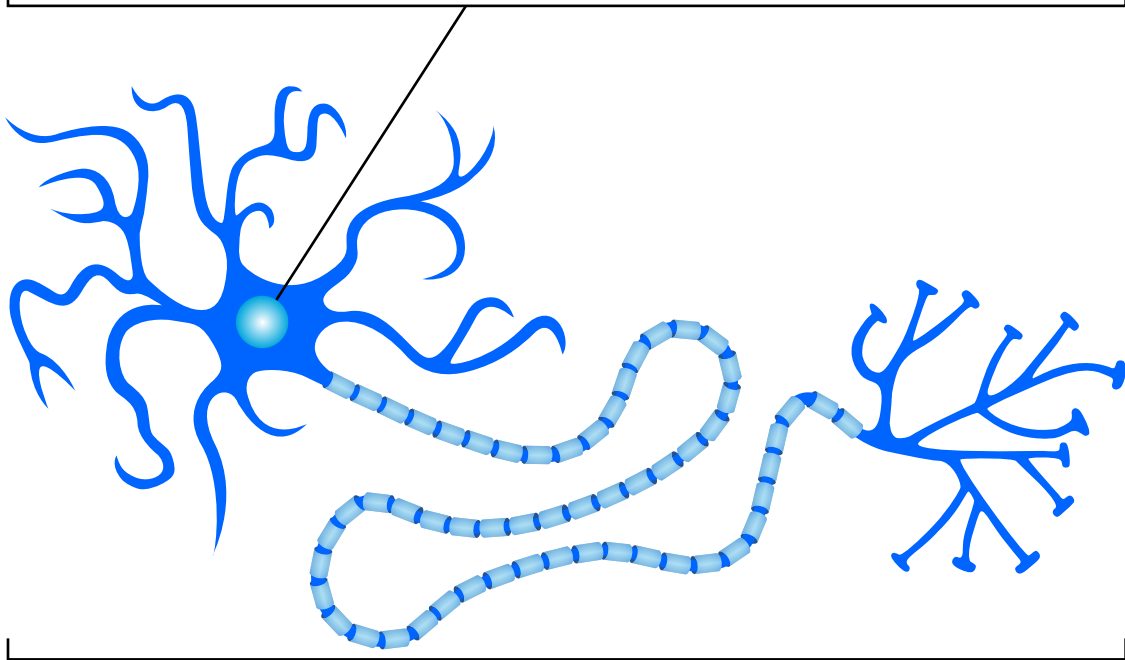
Nuclear Functions of TDP-43

Physiologic

Gene silencing
Enhancement of gene transcription
Nuclear body formation
mRNA splicing
mRNA stabilization
mRNA export

Pathologic

Overproduction of toxic mediators
Loss of vital factors
Disruption of nuclear RNA processing
Nonfunctional critical gene products (ie SMN)
Destabilization of mRNA transcripts (ie NFL)
Retention of mRNA transcripts within nucleus



Cytoplasmic Functions of TDP-43

Physiologic

mRNA transport
mRNA translation
mRNA stabilization
Intracellular signaling (TGF- β pathway)
Response to injury
Protein degradation

Pathologic

Abnormal spatial regulation of mRNA translation
Global disruption of mRNA translation
Premature degradation of mRNA transcripts
Inhibition of TGF- β -mediated signaling
Inability to undergo repair or regeneration
Accumulation of misfolded proteins