Published in final edited form as:
Curr Opin Neurol. 2008 December ; 21(6): 693–700. doi:10.1097/WCO.0b013e3283168d1d.

The role of TDP-43 in amyotrophic lateral sclerosis and frontotemporal dementia

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Abstract

Purpose of review—We examine current evidence that the TAR DNA binding protein, TDP-43, plays a pathogenic role in both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

Recent findings—TDP-43 was recently identified as the major pathological protein in sporadic ALS and in the most common pathological subtype of FTD, frontotemporal lobar degeneration with ubiquitinated inclusions (FLTD-U). In these conditions, abnormal C-terminal fragments of TDP-43 are ubiquitinated, hyperphosphorylated and accumulate as cellular inclusions in neurons and glia. Cells with inclusions show absence of the normal nuclear TDP-43 localization. Recently, missense mutations in the gene encoding TDP-43 have been identified in patients with sporadic and familial ALS.

Summary—The recent discovery of pathological TDP-43 in both ALS and FTLD-U confirms that these are closely related conditions within a new biochemical class of neurodegenerative disease, the TDP-43 proteinopathies.

Keywords

TDP-43; amyotrophic lateral sclerosis; frontotemporal dementia; FTLD-U

Introduction

There is growing evidence that amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are closely related conditions with overlapping clinical, genetic and neuropathological features [1]. The recent identification of the transactive response (TAR) DNA binding protein with M_r 43 kDa (TDP-43) as the major pathological protein, in both ALS and the most common pathological subtype of FTD (frontotemporal lobar degeneration with ubiquitinated inclusions, FTLD-U) [2••,3••], provides the strongest evidence to date that these conditions are part of a clinicopathological spectrum of disease. Furthermore, this discovery

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provides important new insights into the pathogenesis of these conditions and the potential for the development of new diagnostic tests and therapies.

TDP-43 is the pathological protein in ALS and FTLD-U

One of the most characteristic neuropathological features of ALS is the presence of ubiquitin-immunoreactive (ub-ir) neuronal cytoplasmic inclusions (NCI) in the degenerating motor neurons [4]. A significant proportion of ALS patients develop cognitive deficits, often with prominent frontal lobe features [5], and are found to have additional ub-ir NCI and neurites in the frontotemporal neocortex and hippocampus [6,7]. Similar ub-ir, tau- and α-synuclein-negative cortical pathology (FTLD-U) is now recognized to also be the most common pathological substrate for clinical FTD, in the absence of motor features [8,9]. Until recently, it was uncertain whether FTLD-U represented a single disease process, or if it included a number of discrete entities in which the ubiquitinated protein was different. The recognition of several identifiable subtypes of FTLD-U pathology, each with relatively specific clinical and molecular genetic correlations, was initially interpreted as suggesting that FTLD-U was a heterogeneous collection of diseases [10,11]. However, this issue was finally resolved, in late 2006, when two groups independently identified the pathological protein in both FTLD-U and ALS as being TDP-43 [2••,3••].

In those initial studies, immunoblot analysis performed on the high M_r insoluble protein fraction from postmortem FTLD-U brain tissue identified disease specific bands that were excised and analyzed by liquid chromatography-mass spectrometry [2••,3••]. The resulting peptides were found to correspond to amino acid sequences in the C-terminal region of TDP-43. Commercially available antibodies against TDP-43 were found to consistently label the ub-ir inclusions in cases of FTLD-U and also ALS (Figure 1), but not the characteristic inclusions in a variety of other neurodegenerative conditions. An interesting observation, with possible functional implications, was the fact that neurons that contained TDP-43 inclusions showed an absence of the normal diffuse nuclear staining pattern [3••]. In addition to the normal 43 kDa band, TPD-43 immunoblot analysis of FTLD-U and ALS tissue, demonstrated disease specific bands at ~25 and 45 kDa, as well as a high M_r smear (Figure 2). The pathological proteins were shown to represent abnormal C-terminal fragments of TDP-43 that were ubiquitinated and phosphorylated.

A number of subsequent studies have confirmed that most clinical and pathological subtypes of FTLD-U and ALS are characterized by TDP-43 immunoreactive inclusions [12••,13•,14, 15••,16,17]. Moreover, by virtue of its greater sensitivity and specificity, TDP-43 immunohistochemistry has proved to be a powerful new tool for investigating the neuropathology of these conditions, demonstrating changes that were not previously recognized. These include TDP-43-immunoreactive cytoplasmic inclusions in glial cells of presumed oligodendroglial lineage [2••,15••,16,18•], granular neuronal cytoplasmic “pre-inclusions” [19], dystrophic neurites in the CA1 region in cases with hippocampal sclerosis [20] and more extensive extramotor pathology in cases of ALS, with and without dementia [19]. Antibodies raised against phosphorylated epitopes of TDP-43 have proven to be even more sensitive and specific, as they only identify the pathological forms of the protein [21•] while the use of C-terminal and N-terminal specific antibodies has suggested that the pathological inclusions in different populations of neurons may be composed of different forms of TDP-43 [22•]. As a result, FTLD-U and ALS are now recognized as representing a clinicopathological spectrum within a new biochemical class of neurodegenerative disease, the TDP-43 proteinopathies.

Normal function of TDP-43 in nervous system

TDP-43 is a 414 amino acid nuclear protein that is encoded by the *TARDBP* gene on human chromosome 1p36.2. It is highly conserved and ubiquitously expressed in a variety of tissues including brain [23]. TDP-43 contains 2 RNA-recognition motifs (RRM1: ~aa 106–175 and RRM2: ~aa 191–262) and a glycine-rich C-terminal region (~aa 274–413) that allow it to bind single stranded DNA, RNA and proteins [23,24]. It was initially cloned as a human protein capable of binding to the TAR DNA of human HIV-1, where it acts as a transcription repressor [25]. It was subsequently identified as part of a complex involved in splicing the cystic fibrosis transmembrane conductance regulator gene [23] and also the apoA-II gene [26]. The exon skipping and splicing inhibitory activity requires the glycine-rich C-terminal domain that binds to several members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family [24,27]. TDP-43 has also been shown to act as a scaffold for nuclear bodies through an interaction with survival motor neuron protein [28]. It may also be involved in mRNA stability, microRNA biogenesis, apoptosis and cell division [29•]. In the brain, TDP-43 is normally localized to the nucleus of neurons and some glial cells [3••]. Although, its physiological function in the nervous system is not currently known, one recent study has suggested it may act as a neuronal activity-response factor, involved in the regulation of neuronal plasticity [30].

The spectrum TDP-43 proteinopathies

Although the initial reports suggested that pathological TDP-43 is both a specific and sensitive marker of all subtypes of FTLD-U and ALS [2••,3••,13•], subsequent studies have identified some important exceptions. While the vast majority of sporadic FTLD-U cases are found to be TDP-43-positive, most large series have identified a small proportion in which the ub-ir pathology is negative [12••,20]. Two recent papers have provided detailed description of these “atypical” cases (aFTLD-U), which represented 10 – 20 % of all FTLD-U in the respective series [31•,32]. In contrast to TDP-43-positive cases, all aFTLD-U cases were sporadic with very early onset FTD, characterized by severe progressive psychobehavioural abnormalities in the absence of significant aphasia, cognitive-intellectual dysfunction or motor features. The neuropathology consisted of NCI and unique neuronal intranuclear inclusions that were only reactive for ubiquitin. Based on the unusual and highly consistent clinical phenotype and neuropathology, the authors suggested that aFTLD-U represents a newly recognized and specific disease entity.

In familial FTLD-U, different patterns of TDP-43 pathology have been found to correlate with most of the known genetic causes, including mutations in the genes encoding progranulin and valosin-containing protein and in families with FTD and MND linked to chromosome 9p21-13 [12••,14,17]. An exception is FTD linked to chromosome 3, caused by a mutation in the gene encoding the charged multi-vesicular body protein gene (*CHMP2B*), which is characterized by ub-ir NCI in hippocampal granule cells, that are not reactive for TDP-43 [12••,33•].

In ALS, TDP-43 positive inclusions, in both lower motor neurons and glia, are a consistent feature of all sporadic cases and familial cases without *SOD1* mutations [15••,16]. However, the absence of immunohistochemical or biochemical evidence of pathological TDP-43 in human cases and animal models with *SOD1* mutations suggests that neurodegeneration in these cases may have a different pathogenesis [15••,16,34•].

A number of recent studies have also raised questions about the disease specificity of TDP-43 pathology by demonstrating some degree of positivity in a variety of conditions, outside the usual spectrum of FTD and ALS. TDP-43 immunoreactivity is reported to be a consistent feature of ALS-parkinsonism-dementia complex of Guam [35•,36•] and present in a significant proportion of cases of hippocampal sclerosis dementia [12••,37••,38], classical Pick’s disease [39], corticobasal degeneration [40], Alzheimer’s disease [37••,40,41], Parkinson’s disease

and dementia with Lewy bodies [41,42]. In most of these conditions, the TDP-43 pathology is anatomically restricted to mesial temporal structures, shows only partial co-localization with the other pathological changes and is of uncertain clinical relevance [37••,38–42].

It is anticipated that our understanding of the spectrum of TDP-43 proteinopathies will continue to evolve as more cases and additional conditions are examined. Rather than diminish the importance of this protein in the pathogenesis of ALS and FTD, this new information will help to clarify the relationship between different ALS and FTD subtypes and their relationship with other neurodegenerative conditions.

Mutations in *TARDBP* cause ALS but not dementia

Rare pathogenic missense mutations and multiplications have been identified in genes encoding the major constituents of the pathological deposits in several neurodegenerative diseases [43–45]. The gene encoding TDP-43 (*TARDBP*) therefore represents an excellent candidate for causing or increasing the risk to develop a disease in the spectrum of TDP-43 proteinopathies. Initial sequence analyses of *TARDBP* in patients with sporadic FTD and ALS, as well as familial FTD, failed to identify mutations [46]. Similarly, no evidence of genetic variation in *TARDBP* increasing risk for FTD or ALS was observed [46–48]. However, subsequent large population-based *TARDBP* mutation screenings, by us and by others, have identified 16 different missense mutations in 19 genealogically unrelated ALS patients, which were absent in healthy controls [49•,50•,51••,52,53•] (Figure 3). Eight mutations have been found in familial ALS patients [49•,50•,51••,52,53•], while eight others were identified in patients with sporadic ALS [50•,51••]. Mutations c.1077G>A (p.A315T), c.1009A>G (p.M337V) and c.1278G>A (p.A382T) were identified in two families each and haplotype sharing studies supported a common genetic origin for mutations p.M337V and p.A382T. *TARDBP* mutations were not restricted to Caucasians ALS populations; mutation c.892G>A (p.G298S) was identified in a Chinese family [53•] and c.1028A>G (p.Q343R) in a Japanese family [52].

Depending on the study, the overall *TARDBP* mutation frequency in ALS has ranged from 3% to 6%, with the exception of the initial report by Sreedharan et al. [51••], in which a much lower *TARDBP* mutation frequency of 0.6% (3/526) was identified. Both population-based studies in which sporadic mutation carriers were identified, described comparable *TARDBP* frequencies for sporadic and familial ALS [50•,51••]; however mutation analyses in a large clinical series of sporadic ALS patients of European descent did not identify mutations [54] and other studies have identified *TARDBP* mutations exclusively in familial ALS [49•,52, 53•]. Without supportive functional data, the pathogenic character of each of the *TARDBP* mutations identified in a single sporadic ALS patient remains uncertain.

The clinical phenotype of *TARDBP* mutation carriers resembles classical adult onset ALS. Based on the current published literature, the mean onset age for mutation carriers was 56.3 ± 12.1 years (N=30; range 30–83 years) with similar onset ages in familial (N=22; 55.8 ± 12.1 years) and sporadic (N=8; 57.5 ± 12.8 years) cases [49•,50•,51••,52,53•]. The mean duration of disease was 4.3 ± 3.6 years (N=18; range 1–12 years). Even within a single family, the age of onset varied significantly (by as much as 35 years) [53•], suggesting that additional genetic and/or environmental factors determine the disease expression of *TARDBP* mutations. Thirty-three percent of mutation carriers (11/33) had bulbar onset and 67% (22/33) limb-onset ALS, a distribution comparable to non-*TARDBP* mutation carriers [55,56]. Interestingly, despite the common co-occurrence of cognitive symptoms in ALS patients [5], none of the *TARDBP* mutation carriers identified to date, have had a personal or family history of dementia. In agreement with this finding, *TARDBP* mutations have not been identified in patients with FTD, FTD-ALS or AD, characterized by TDP-43 pathology [50•,53•].

Thus far, pathological examination has only been performed in two families with *TARDBP* mutations [52,53•]. The neuropathological changes in patients from both families were characteristic of ALS, with motor neuron loss, gliosis and the presence of Bunina bodies. As expected, TDP-43-positive NCIs and glial cytoplasmic inclusions were observed in the anterior horn cells of the spinal cord and in various other regions of the central nervous system. In one family the authors suggested a higher frequency of TDP-43-positive "pre-inclusions" compared to other patients with sporadic ALS [53•]. Biochemical analyses of TDP-43 protein extracted from the spinal cord of the autopsied case carrying the p.Q343R mutation showed elevated levels of the abnormal molecular-weight fragments of ~25 and 45kDa, that were previously observed in sporadic ALS and in *SOD-1* negative familial ALS, suggesting *TARDBP* mutations may accelerate the production of these fragments [52]. Whether these neuropathological findings are characteristic of all *TARDBP* mutation carriers needs further confirmation.

All but one of the reported mutations cluster in *TARDBP* exon 6, encoding the highly conserved C-terminal region of TDP-43 (Figure 3). Four mutations involve glycine residues, in close proximity of each other, in the glycine-rich region, while the other mutations affect conserved residues in the remaining portion of the C-terminal domain. No clustering of familial versus sporadic mutations in specific regions of the gene has been observed and no effect of the location of the mutations on the age of onset or disease duration has been demonstrated.

Role of TDP-43 in neurodegeneration

Little is currently known about the pathogenic role of *TARDBP* mutations or TDP-43 pathology in neurodegeneration. Analogous to other neurodegenerative diseases, characterized by the accumulation of mis-folded protein, the critical step will be to determine whether disease results from a loss-of-function, a toxic gain-of-function or both.

The abnormal localization of TDP-43 to the cytoplasm in affected neurons in FTD and ALS, irrespective of the presence of a genetic mutation, suggests a pathogenic mechanism associated with the loss of the normal nuclear TDP-43 function in regulating transcription, splicing and mRNA stability [29•,57]. In support of this hypothesis, loss of TDP-43 in human cells has been shown to induce morphological nuclear defects and increased apoptosis [58••]. Alternatively, sequestration of TDP-43 in cellular inclusions may induce a toxic gain-of-function, independent of the basic biological role of TDP-43. A study in which TDP-43 was overexpressed in yeast suggested that only aggregating forms of TDP-43 were toxic, although the toxicity depended on an intact RNA recognition motif [59•]. Factors that affect the normal intracellular trafficking of TDP-43, between the cytoplasm and nucleus, may predispose to both the formation of abnormal aggregates (inclusions) and the loss of nuclear localization [60•].

The identification of *TARDBP* mutations in ALS has provided important clues to the possible pathogenic mechanisms involved in TDP-43 proteinopathies. The clustering of mutations in exon 6, that encodes the highly conserved C-terminal domain, suggests that *TARDBP* mutations may interfere with the normal protein-protein interactions of TDP-43, affect its transport through the nuclear pore or influence its exon skipping or transcriptional repression activity [50•,51••]. Numerous potential phosphorylation sites have been predicted to occur in the C-terminal region of TDP-43 and some mutations, especially the six substitutions to serine and threonine residues, may increase phosphorylation and aggregation. A single mutation c. 640A>T (p.D169G) was identified in *TARDBP* exon 4 encoding RRM1 [50•]. This mutation may abrogate the RNA binding to this region; however, because it lies outside of exon 6 and lacks evidence of segregation with disease, it may also represent a rare benign sequence variant. Finally, a study of mutant TDP-43 in cell culture and *in vivo* revealed increased generation of

detergent-insoluble TDP-43 fragments that could aggregate and cause a toxic gain-of-function [50•,51••]. Given the multifaceted role of TDP-43, multiple disease mechanism may well be involved.

Conclusions

The identification of pathological TDP-43 in FTLD-U and ALS confirms that these conditions are part of a disease spectrum with a common underlying biochemical mechanism. This insight has helped to clarify the relationship among the various genetic, clinical and pathological subtypes of FTD and ALS and has suggested possible mechanistic links with other neurodegenerative disorders. A better understanding of the role of TDP-43 in neurodegeneration will be crucial to the development of targeted therapeutic strategies for these conditions. Central to this process will be the use of experimental animals and cell systems, expressing pathogenic mutations in *TARDBP* or FTLD-U causing genes. The recent identification of elevated plasma levels of TDP-43, in some patients with neurodegenerative disease [61•], supports the possible use of TDP-43 as an *in vivo* biomarker to aid in diagnosis and monitoring the effects of therapy. In summary, the discovery that TDP-43 plays a central role in the pathogenesis of FTD and ALS has been a major advance towards the effective management of these devastating neurodegenerative conditions.

Acknowledgments

This work was supported by funding from the Canadian Institutes for Health Research (#135169 and 179009), the Pacific Alzheimer Research Foundation (#C06-01) and the National Institutes of Health (P50 AG16574).

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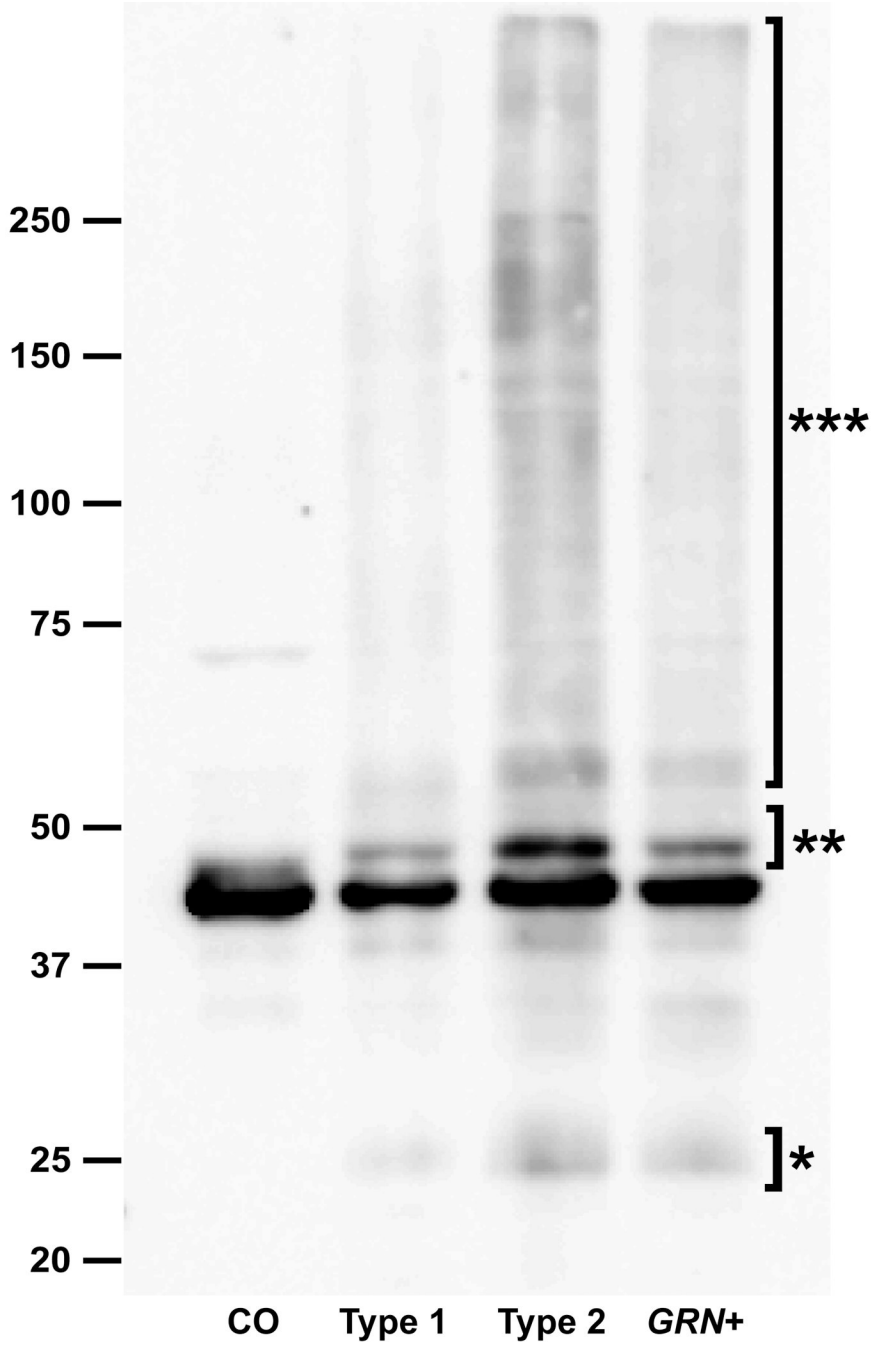

Fig. 1. TDP-43 immunohistochemistry in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) and amyotrophic lateral sclerosis (ALS)

A polyclonal antibody against TDP-43 labels neuronal inclusions in (a) neocortex and (b) hippocampus in FTLD-U and (c) lower motor neurons in ALS. Note that cells with inclusions show loss of normal diffuse nuclear staining. TDP-43 immunohistochemistry. Scale bar; (a,b) 20 μm, (c) 15μm.

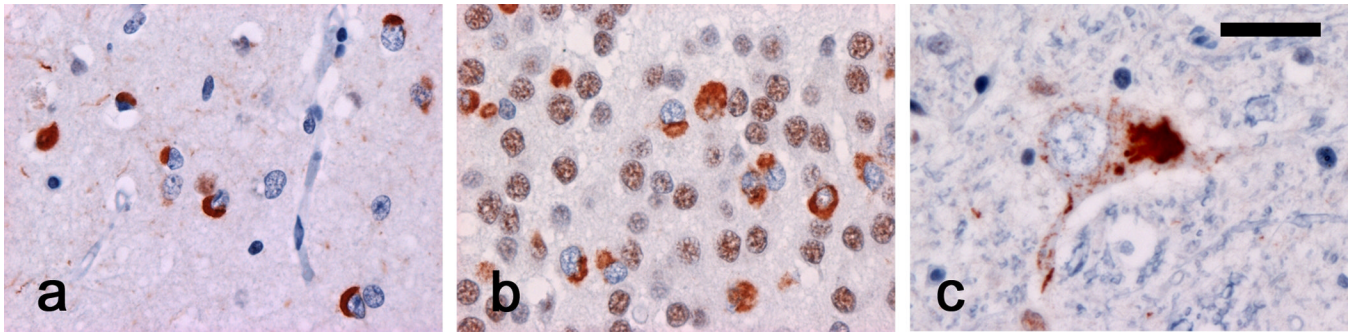

Fig. 2. Biochemical analysis of pathologic TDP-43 in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U)

Immunoblot analysis of urea fraction from post mortem FTLD-U brain tissue using anti-TDP-43 antibody demonstrates pathological bands at ~25 kD (*), ~45 kD (**) and a high M_r smear (***). CO, control; type 1, sporadic FTLD-U with type 1 pathology; type 2, sporadic FTLD-U with type 2 pathology; *GRN+*, familial FTLD-U with *progranulin* mutation and type 3 pathology [11]. A similar immunoblot pattern is seen in studies of post mortem spinal cord tissue from cases of amyotrophic lateral sclerosis. Previously published and reprinted with permission [62].

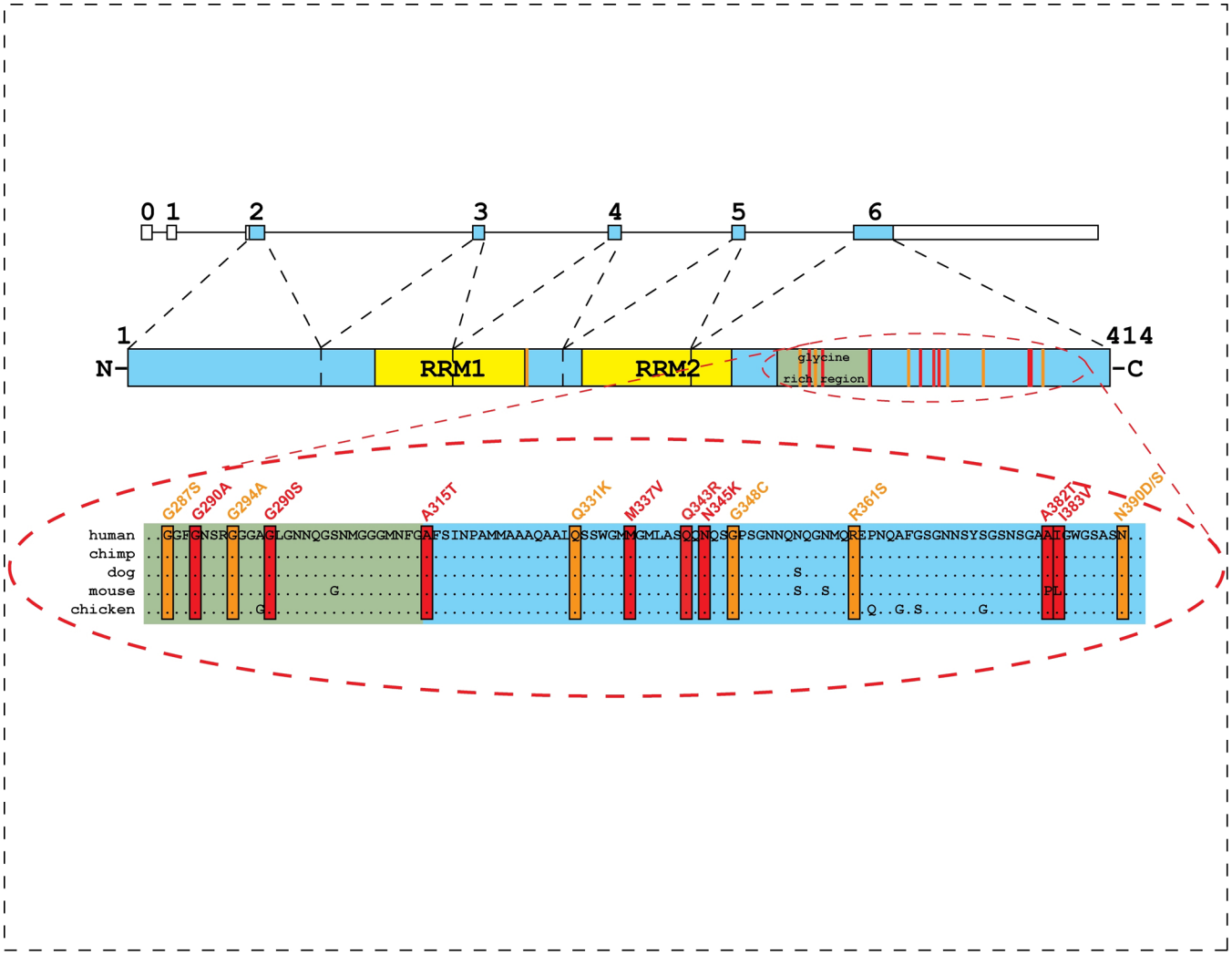

Fig. 3. Missense mutations in the gene encoding TDP-43 (*TARDBP*) cause ALS

Schematic overview of the TDP-43 gene (*TARDBP*) and protein structure with locations of conserved domains (protein numbering according to the largest isoforms NP_031401.1) (top). Protein sequence alignment shows strong conservation in the C-terminal region of TDP-43 (bottom). Colored boxes indicate the position of *TARDBP* mutations identified in sporadic (orange) and familial (red) ALS patients. RRM= RNA recognition motif.