

TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis

Edor Kabashi^{1,6}, Paul N Valdmanis^{1,6}, Patrick Dion¹, Dan Spiegelman¹, Brendan J McConkey², Christine Vande Velde¹, Jean-Pierre Bouchard³, Lucette Lacomblez⁴, Ksenia Pochigaeva⁴, Francois Salachas⁴, Pierre-Francois Pradat⁴, William Camu⁵, Vincent Meininger⁴, Nicolas Dupre^{1,3} & Guy A Rouleau¹

Recently, TDP-43 was identified as a key component of ubiquitinated aggregates in amyotrophic lateral sclerosis (ALS), an adult-onset neurological disorder that leads to the degeneration of motor neurons. Here we report eight missense mutations in nine individuals—six from individuals with sporadic ALS (SALS) and three from those with familial ALS (FALS)—and a concurring increase of a smaller TDP-43 product. These findings further corroborate that TDP-43 is involved in ALS pathogenesis.

Prominent ubiquitin-positive inclusion bodies are considered a pathological hallmark of many neurodegenerative diseases, including ALS¹. The composition of these inclusion bodies that aggregate in individuals with ALS has been elusive, but the TAR-DNA binding protein TDP-43 (encoded by *TARDBP*) was recently identified as the primary component^{2–4}. Amino acid changes in this protein, if present, may hasten the aggregation process. To investigate the possible role of TDP-43 in ALS, we screened a panel of SALS and FALS cases for *TARDBP* mutations.

The *TARDBP* gene contains six exons (five coding) and is located on chromosome 1p36.22 (Fig. 1a). The coding region of *TARDBP* was screened in its entirety in 200 individuals with ALS (80 FALS, 120 SALS) from France and Quebec (Supplementary Table 1 online) and 185 controls matched for age and ethnicity (Supplementary Note and Supplementary Methods online). We identified eight distinct heterozygous missense mutations in nine individuals (Fig. 1). These variants were identified in six SALS and three FALS cases (Table 1) and were not present in the initial 185 controls or in 175 additional controls (720 total chromosomes). None of these affected individuals had a personal or family history of frontotemporal dementia (FTD). We were able to show the segregation of the A315T substitution, which was present in both an affected mother and her affected son, but not in three of their unaffected relatives (Fig. 1c). We further identified another variant (A382T) in two familial cases; however, no additional

family members from these families were available. Although we cannot be certain that the two families are related, haplotype analysis indicates that they share alleles for six microsatellite markers surrounding *TARDBP* (Supplementary Table 2 online). Notably, the asparagine residue at codon 390 was altered in two different individuals: once to aspartate, and once to serine (Table 1). This is relevant, as one isoform of TDP-43 that terminates at codon 390 was previously detected in individuals with ALS⁵. Finally, we observed two silent changes in two individuals with FALS (Ala66) and one individual with SALS (Ala315). Unlike the missense mutations, these silent changes are less likely to be pathogenic, although they may influence the expression or splicing of the *TARDBP* mRNA. In the 185 controls sequenced for the entire gene, we identified one variant, A90V, in one control individual, and we did not identify any silent changes. Another group also did not identify any mutations in 173 individuals with ALS and 237 individuals with FTD from Belgium⁶. Thus, *TARDBP* does not have a high degree of overall sequence variation. The 4.5% of missense mutations in our sampled ALS cases is significantly higher than that found in controls (Fisher's exact test, $P = 0.027$). This profile of mutations identified for *TARDBP* is similar to what has been observed for *ANG* (angiogenin), another gene associated with ALS⁷.

Of the seven altered amino acids, four were fully conserved across eight species examined. Asp169 and Ala315 were conserved in seven species; whereas Ala382 was conserved in six (Supplementary Fig. 1 online). Five of the eight missense mutations were predicted by at least one of three bioinformatics prediction programs to have a deleterious effect on protein structure or function (Supplementary Table 3 online). However, the control A90V variant was predicted to have no effect on protein function, and it had the least severe or second-to-least severe score compared with the mutations found in individuals with ALS.

The D169G substitution is in the first RNA-binding motif of TDP-43 and may abrogate this RNA binding. The G348C variant introduces a cysteine to this region and may markedly increase the propensity for aggregation through the formation of intermolecular disulfide bridges. Altogether, we found seven mutations that affected the C terminus of TDP-43 (Fig. 1), a region which is predicted to be disordered, and which contains several instances of Phe-Gly and Trp-Gly motifs (Supplementary Fig. 2 online). Similar Phe-Gly motifs occurring in nuclear pore proteins mediate interactions between disordered protein regions⁸; a similar mechanism likely occurs between the disordered regions of TDP-43 and one of its interacting partners, heterogeneous nuclear ribonucleoprotein A/B (hnRNP)⁹. Numerous potential phosphorylation sites are also predicted to occur in the C-terminal region.

¹Center of Excellence in Neuromics, Centre Hospitalier de l'Université de Montréal, and Department of Medicine, University of Montreal, Montreal, Quebec H2L4M1, Canada. ²Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario N2L 3G1, Canada. ³Faculty of Medicine, Laval University, Centre Hospitalier Affilié Universitaire de Québec – Enfant-Jésus Hospital, Québec G1J1Z4, Canada. ⁴Fédération des maladies du système nerveux, Division Paul Castaigne, Hôpital de la Salpêtrière, Paris 75651, France. ⁵Unité de Neurologie Comportementale et Dégénérative, Institute of Biology, Montpellier 34967, France. ⁶These authors contributed equally to this work. Correspondence should be addressed to G.A.R. (guy.rouleau@umontreal.ca).

Received 10 December 2007; accepted 5 March 2008; published online 30 March 2008; doi:10.1038/ng.132

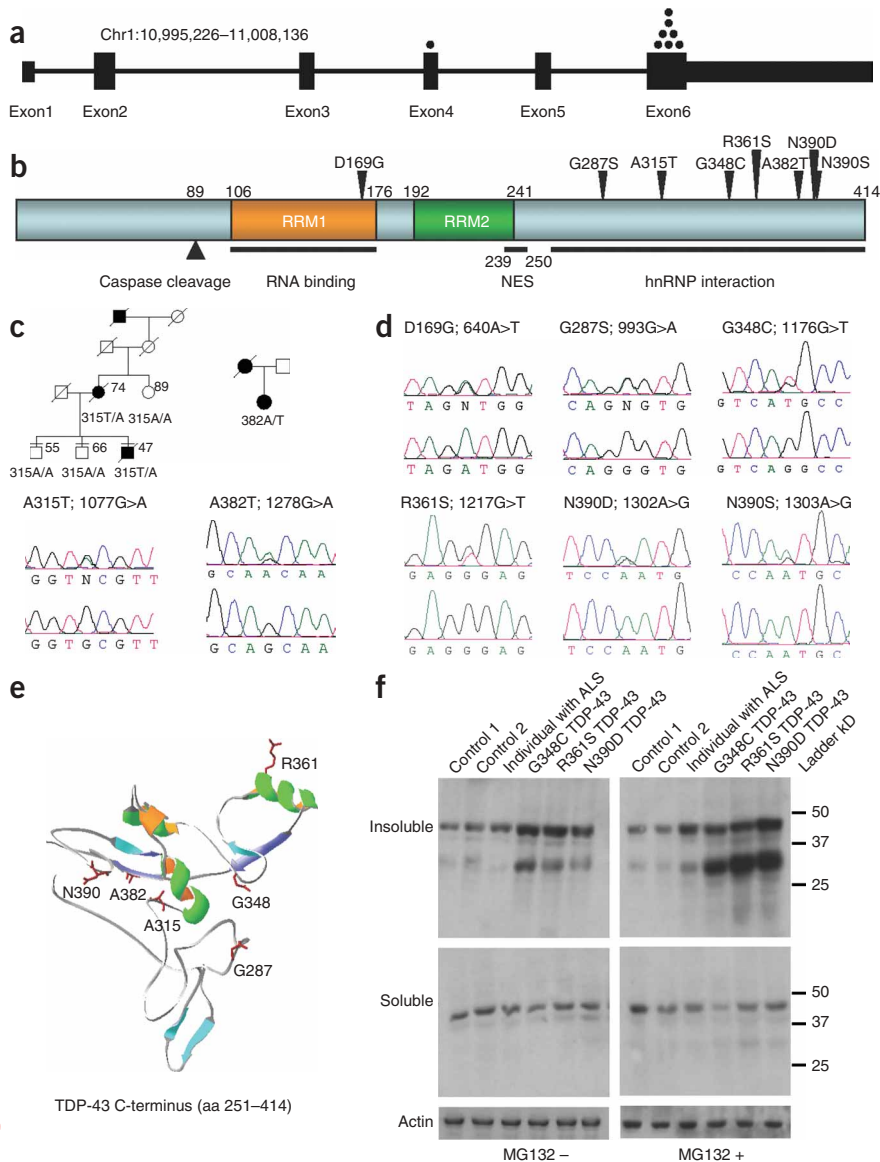


Figure 1 *TARDBP* mutations in individuals with ALS and their effect on the translated protein. **(a)** Gene structure of *TARDBP* indicating untranslated exons (medium-height bars) and coding exons (thick bars). Circles above the exon indicate the location of each identified mutation in individuals with ALS. **(b)** Linear protein structure of TDP-43 indicating the RNA-recognition motifs (RRM1 and RRM2). Regions responsible for RNA binding, the nuclear export sequence (NES) and binding with hnRNP are indicated by horizontal bars below the protein. Amino acid changes are shown above the protein with arrowheads indicating their position. **(c)** Pedigrees for selected individuals with *TARDBP* mutations. Mutations are indicated using single letter amino acid code and their sequence traces are shown below. Numbers above individuals indicate the age of symptom onset or, in the case of unaffected individuals, the age when collected. **(d)** Sequence traces of the identified mutations in individuals with SALS. **(e)** Three-dimensional modeling of the C-terminal region (amino acids 250–414) of TDP-43 in the wild-type form. The positions of the six affected residues are indicated. Note that the altered serine residue is the one shown at position 390. **(f)** Protein lysates from three individuals with ALS expressing TDP-43 mutants show the accumulation of a smaller product of TDP-43 protein (~28 kDa), mainly in a detergent-insoluble fraction. The expression of this shorter form was substantially lower in two unaffected individuals (controls 1 and 2) and in an individual with ALS without a mutation in *TARDBP*. This product is likely degraded by the ubiquitin-proteasome pathway, as proteasome inhibition by MG132 leads to its accumulation.

and mass spectroscopy data has shown that this species corresponds to a minimal region of amino acids 252–414 from TDP-43, where most of the substitutions we identified are located⁴.

The TDP-43 protein was initially identified as capable of binding the TAR DNA sequence motifs of HIV¹⁰, and it was also shown to bind repeat stretches of DNA and RNA and act as a transcriptional repressor¹¹. Further, TDP-43 has splicing inhibitory activity, which can lead to exon skipping through protein–protein interactions⁷. TDP-43 has also been implicated in the splicing of the cystic fibrosis transmembrane receptor (CFTR)^{9,12} by recruiting hnRNP, which is involved in mRNA biogenesis⁹. Finally, TDP-43, through interaction with the survival motor neuron protein (SMN), may also act as a scaffold for nuclear bodies¹³.

Immunohistochemical analyses of a number of neurological disorders, including ALS, FTD and Alzheimer's disease¹⁴, have shown that TDP-43 protein is present in inclusion bodies. Further, disruption of the ubiquitin-proteasome pathway and autophagy lead to aberrant phosphorylation and ubiquitination of TDP-43 and its accumulation in detergent-insoluble aggregates¹⁵. However, its contribution to disease predisposition, onset or progression remains to be elucidated.

The mutations we have identified that affect the C-terminal region of TDP-43 may influence protein–protein interaction (for example, with hnRNP A/B), transport through the nuclear pore, or exon

Most of the mutations identified here were predicted to increase TDP-43 phosphorylation, particularly as five of these resulting substitutions are to threonine or serine residues (**Supplementary Table 4** online). This could potentially interfere with protein interactions or transport through the nuclear pore complex and lead to progressive accumulation of the aggregates seen in individuals with ALS^{2–4}. Indeed, lymphoblastoid cell lines derived from individuals treated with the proteasome inhibitor MG132, but not those from similarly treated controls, clearly show the accumulation of a detergent-insoluble TDP-43 protein product of ~28 kDa (**Fig. 1f** and **Supplementary Fig. 2a**) and thus confirm the increased aggregation property of these TDP-43 mutants (**Supplementary Methods**). Further confirming the increased aggregation property of these TDP-43 mutants, the amount of TDP-43 was unchanged in the fraction containing only the soluble proteins (**Fig. 1f**). The inhibitory activity of MG132 on the proteasomal machinery was confirmed using HSP70, which rapidly accumulates upon such inhibition (**Supplementary Fig. 2b**). This smaller product has previously been observed in conjunction with the presence of TDP-43–positive aggregates in individuals with ALS^{4,5},

Table 1 Characterization of individuals with *TARDBP* mutations

Variant	Amino acid change	Nucleotide change	Exon	Origin	Family history of ALS	Sex	Age of onset (years)	Duration (months)	Site of onset	El Escorial criteria	UMN signs	LMN signs	Cognitive impairment signs
1	D169G	640A>G	4	France	No	Female	56	40 ^a	Spinal	Probable	Yes	Yes	None
2	G287S	993G>A	6	France	No	Male	65	63 ^a	Nulbar	Definite	Yes	Yes	None
3 ^b	A315T	1077G>A	6	France	Yes	Female	74	48	Spinal	Probable	Yes	Yes	None
3 ^b	A315T	1077G>A	6	France	Yes	Male	47	132	Spinal	Probable	Mild	Predominant	None
4	G348C	1176G>T	6	France	No	Female	30	84	Spinal	Definite	Yes	Yes	Apathy; major anxiety
5	R361S	1217G>T	6	France	No	Male	55	60 ^a	Spinal	Definite	Yes	Yes	None
6	A382T	1278G>A	6	France	Yes	Female	55	28	Bulbar	Definite	Mild	Yes	None
6	A382T	1278G>A	6	France	Yes	Female	57	73	Spinal	Definite	Mild	Predominant	None
7	N390D	1302A>G	6	Quebec	No	Male	53	35	Spinal	Definite	Yes	Yes	None
8	N390S	1303A>G	6	France	No	Male	64	23	Bulbar	Probable	Yes	Yes	Agitation

^aIndividual is living. ^bMother-son pair.

skipping and splicing inhibitory activity. Thus, these mutations may influence the proper function or transport of TDP-43. They may also cause a toxic gain of function through novel interactions or intracellular aggregation, particularly of the ~28 kDa species, which showed accumulation in the ALS cases with *TARDBP* mutations (Fig. 1f). The identification of missense mutations in *TARDBP* is a link between the genetic predisposition for ALS and the presence of TDP-43 in intracellular aggregates of individuals with ALS.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We would like to thank all the families involved in this study. We also thank M. Benard, I. Thibault and P. Provencher for sample collection and organization, M. D'Amour and D. Brunet for providing access to their patients and A. Dyck and J. St-Onge for technical support. G.A.R. is funded by the Canadian Institutes of Health Research (CIHR), Muscular Dystrophy Association USA and ALS Association (ALSA), E.K. by ALS Canada and CIHR, N.D. by CIHR, P.N.V. by the Fonds de Recherche en Sante Quebec (FRSQ) and V.M. by the Association Francaise contre les Myopathies France (AMF) and the Association pour la Recherche sur la Sclerose Laterale Amyotrophique (ARS).

AUTHOR CONTRIBUTIONS

E.K. and P.N.V. generated the data, conducted the data analysis, wrote the manuscript and led the project; P.D. participated in the data analysis and review of the manuscript; E.K. and P.D. conducted the functional analysis of lymphoblastoid cell lines derived from individuals with ALS; P.N.V. conducted haplotype and performed 3D modelling; D.S. performed sequence and data

analysis; B.J.M. conducted bioinformatic analysis of mutations and reviewed the manuscript; C.V.V. performed functional analysis and reviewed the manuscript. J.-P.B., L.L., K.P., F.S., P.-F.P., W.C., V.M. and N.D. conducted neurological evaluation and family history of individuals with ALS and reviewed the manuscript. G.A.R. conducted neurological evaluation of individuals with ALS, participated in the data analysis, reviewed the manuscript and supervised the project.

Published online at <http://www.nature.com/naturegenetics>
Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions>

- Lansbury, P.T. & Lashuel, H.A. *Nature* **443**, 774–779 (2006).
- Arai, T. *et al. Biochem. Biophys. Res. Commun.* **351**, 602–611 (2006).
- Mackenzie, I.R. *et al. Ann. Neurol.* **61**, 427–434 (2007).
- Neumann, M. *et al. Science* **314**, 130–133 (2006).
- Strong, M.J. *et al. Mol. Cell. Neurosci.* **35**, 320–327 (2007).
- Gijssels, I. *et al. Neurobiol. Aging* (in press).
- Greenway, M.J. *et al. Nat. Genet.* **38**, 411–413 (2006).
- Frey, S., Richter, R.P. & Gorlich, D. *Science* **314**, 815–817 (2006).
- Buratti, E. *et al. J. Biol. Chem.* **280**, 37572–37584 (2005).
- Ou, S.H., Wu, F., Harrich, D., Garcia-Martinez, L.F. & Gaynor, R.B. *J. Virol.* **69**, 3584–3596 (1995).
- Forman, M.S., Trojanowski, J.Q. & Lee, V.M. *Curr. Opin. Neurobiol.* **17**, 548–555 (2007).
- Wang, H.Y., Wang, I.F., Bose, J. & Shen, C.K. *Genomics* **83**, 130–139 (2004).
- Wang, I.F., Reddy, N.M. & Shen, C.K. *Proc. Natl. Acad. Sci. USA* **99**, 13583–13588 (2002).
- Amador-Ortiz, C. *et al. Ann. Neurol.* **61**, 435–445 (2007).
- Filimonenko, M. *et al. J. Cell Biol.* **179**, 485–500 (2007).