Published in final edited form as:

Acta Neuropathol. 2009 November ; 118(5): 633–645. doi:10.1007/s00401-009-0571-7.

TARDBP 3'-UTR variant in autopsy-confirmed frontotemporal lobar degeneration with TDP-43 proteinopathy

Michael A. Gitcho,

Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA

Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA

Eileen H. Bigio,

Alzheimer Disease Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Manjari Mishra,

Alzheimer Disease Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Nancy Johnson,

Alzheimer Disease Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Department of Psychiatry, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Sandra Weintraub,

Alzheimer Disease Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Department of Cognitive Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Marsel Mesulam,

Alzheimer Disease Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Department of Cognitive Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Rosa Rademakers,

Department of Neuroscience, Mayo Clinic, Jacksonville, FL, USA

Sumi Chakraverty,

Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA

Correspondence to: Nigel J. Cairns, cairns@wustl.edu.

M.A. Gitcho and E.H. Bigio contributed equally to this work.

Conflict of interest statement The authors report no conflicts of interest.

Carlos Cruchaga,

Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA

John C. Morris,

Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA

Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA

Department of Pathology and Immunology, Washington University School of Medicine, Campus Box 8118, 660 South Euclid Avenue, St. Louis, MO 63110, USA

Alison M. Goate, and

Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA

Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA

Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA

Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA

Nigel J. Cairns

Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA

Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA

Department of Pathology and Immunology, Washington University School of Medicine, Campus Box 8118, 660 South Euclid Avenue, St. Louis, MO 63110, USA

Nigel J. Cairns: cairns@wustl.edu

Abstract

Pathogenic mutations in the gene encoding TDP-43, *TARDBP*, have been reported in familial amyotrophic lateral sclerosis (FALS) and, more recently, in families with a heterogeneous clinical phenotype including both ALS and frontotemporal lobar degeneration (FTLD). In our previous study, sequencing analyses identified one variant in the 3'-untranslated region (3'-UTR) of the *TARDBP* gene in two affected members of one family with bvFTD and ALS and in one unrelated clinically assessed case of FALS. Since that study, brain tissue has become available and provides autopsy confirmation of FTLD-TDP in the proband and ALS in the brother of the bvFTD-ALS family and the neuropathology of those two cases is reported here. The 3'-UTR variant was not found in 982 control subjects (1,964 alleles). To determine the functional significance of this variant, we undertook quantitative gene expression analysis. Allele-specific amplification showed a significant increase of 22% ($P < 0.05$) in disease-specific allele expression with a twofold increase in total *TARDBP* mRNA. The segregation of this variant in a family with clinical bvFTD and ALS adds to the spectrum of clinical phenotypes previously associated with *TARDBP* variants. In summary, *TARDBP* variants may result in clinically and neuropathologically heterogeneous phenotypes linked by a common molecular pathology called TDP-43 proteinopathy.

Keywords

Frontotemporal lobar degeneration; Frontotemporal dementia; Motor neuron disease; Amyotrophic lateral sclerosis; TDP-43; *TARDBP*; 3'-Untranslated region

Introduction

Mutations in the TAR DNA-binding protein of 43 kDa (*TARDBP/TDP-43*) gene have recently been identified as a novel cause of familial and sporadic amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) [20,26,37,39,55,56,58], and in clinically heterogeneous families with either MND or frontotemporal lobar degeneration (FTLD-MND), or both, within a single family [8]. The deduced 414-amino acid TDP-43 protein contains two ribonucleoprotein (RNP)-binding domains and a glycine-rich region [52,59] (Fig. 1a). This predominant nuclear protein function as a DNA- and RNA-binding molecule that regulates transcription and splicing and is involved in the regulation of cystic fibrosis transmembrane conductance regulator (CFTR) splicing by promoting CFTR exon 9 skipping by binding to the GU-repeated motifs in the polymorphic region near the 3'-splice site of this exon [13–16]. The resulting abnormal splicing is associated with the pathology of cystic fibrosis [13,14,16]. The glycine-rich domain is required for exon-skipping activity [16,59]. TDP-43 was recently found to be involved in exon inclusion of survival of motor neuron (SMN) [10,28], where the loss of function causes spinal muscular atrophy (SMA). *TARDBP* mRNA is ubiquitously expressed with high levels in neocortex, hippocampus, and cerebellum (see Allen Brain Atlas at <http://www.brain-map.org/>). TDP-43 has become the focus of intense scrutiny because it is the major pathological protein of the inclusions of most cases of sporadic and familial FTLD-TDP, ALS/MND, and FTLD-MND [3,18,42,51], and is a co-morbidity in a broadening spectrum of other neurodegenerative diseases [2,23,25,29,32,48,50,62].

TDP-43 was first identified as the major pathological protein of ubiquitin-positive, tau-negative inclusions of frontotemporal lobar degeneration (FTLD-U, also called FTLD with TDP-43 proteinopathy or FTLD-TDP), FTLD with motor neuron disease (FTLD-MND), and ALS/MND [3,17,41–43,50,51]. These disorders are now regarded as different clinical manifestations of the same underlying molecular pathology, namely TDP-43 proteinopathy. The differing clinical phenotypes of these clinically overlapping disorders most likely reflect the selective vulnerability of different segments of the neuraxis to neurodegeneration caused by TDP-43 proteinopathy. The reasons for selective vulnerability are not currently known. The biochemical signature of this proteinopathy includes the following: urea-soluble ubiquitinated protein, hyperphosphorylated TDP-43, and C-terminal fragments of ~25 kDa in affected neocortex, hippocampus, and spinal cord from individuals with ALS/MND, FTLD-MND, and FTLD-TDP [3,30,51]. Defects in the *TARDBP* gene are now recognized as a cause of amyotrophic lateral sclerosis type 10 (ALS10) (see Online Mendelian Inheritance in Man at <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi>). Amyotrophic lateral sclerosis/MND is a neurodegenerative disorder affecting both upper and lower motor neurons and results in fatal paralysis usually within 2–5 years. Most of the variants reported occur within the glycine-rich region, which indicates that this is a functionally important domain in normal cellular metabolism [26,37,39,55,56,58]. The frequency of *TARDBP* mutations in most series is <5% of familial amyotrophic lateral sclerosis (FALS) cases, but several families with an autosomal dominant mode of inheritance with TDP-43 proteinopathy including FTLD-MND and FTLD-TDP remain unexplained. To further explore the contribution of *TARDBP* allelic variants to the spectrum of clinical phenotypes with TDP-43 proteinopathy, we investigated a proband from each 'FTLD' or 'MND' family in which no pathogenic variant had yet been identified and also extended our analysis to include all exons and intronic boundaries and the relatively large 3'-untranslated region (3'-UTR) of the gene. We report here the neuropathology and functional studies of our previously identified variant in the 3'UTR of *TARDBP* [26]. We have identified three cases from two genealogically unrelated families. One patient harboring the variant presented clinically with behavioral variant frontotemporal dementia (bvFTD) and had the pathologic stigmata of FTLD-TDP. To our knowledge, this is the first report of a deceased individual

with clinical bvFTD and a *TARDBP* 3'UTR variant who has had autopsy brain evaluation and confirmation of FTLT-DTP without motor neuron disease. Recently, another variant was described in two patients, one with semantic dementia and ALS and one with a behavioral disorder and ALS [8], but no neuropathology was available in either case. This and other studies support the observation that *TARDBP* mutations may result in clinically and neuropathologically heterogeneous phenotypes.

Patients and methods

Cases

We report the neuropathology of two cases in which we previously identified a 3'UTR variant in *TARDBP* in two families [26]. The cases were identified from the archives of the Alzheimer Disease Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA. For gene expression studies, normal control cases and sporadic and familial FTLT-DTP cases were obtained from the Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA (Table 1). Briefly, cases previously sequenced and identified with progranulin (GRN) or valosin-containing protein (VCP) mutations, or FTLT-MND cases linked to chromosome 9, including those with putative variants in the intraflagellar transport homolog 74 (IFT74) [45] or ubiquitin-associated protein 1 (UBAP1) [54] genes, were excluded. In our previous mutational analysis, we identified the variant described here in two families [26]. Subsequent to that report, we have identified the same variant in the proband's brother and autopsy material has become available, the subject of this report.

Neuropathology

The brain was sliced fresh, and slabs were fixed for 30 h at 4°C in 4% paraformaldehyde, cryoprotected in buffer with increasing sucrose gradients to 40%, and stored in 40% sucrose with sodium azide. Tissue blocks from this tissue were processed and paraffin-embedded and sections were cut at 5 µm for histochemistry and immunohistochemistry. Multiple regions were examined, including frontal, temporal, parietal, and occipital lobes, thalamus, striatum, basal ganglia including the nucleus basalis of Meynert, amygdala, hippocampus, midbrain, pons, medulla oblongata, and the upper cervical spinal cord. Histochemical stains included: hematoxylin and eosin, Luxol fast blue-hematoxylin with periodic-acid-Schiff; cresyl fast violet; thioflavin S; and modified Bielschowsky and Gallyas silver impregnations. Immunohistochemistry was performed using the following antibodies: Aβ (1:100,000, mouse monoclonal antibody (MAB), 10D5; gift of Eli Lilly, Indianapolis, IN, USA, and mouse MAB, 4G8, 1:4,000, Senetek, St Louis, MO, USA); phosphorylated tau, (mouse MAB, PHF-1, 1:500, and mouse MAB, Alz-50, 1:50, kindly supplied by Dr Peter Davies, Albert Einstein Medical School, Bronx, NY, USA; and AT8, mouse MAB, 1:1,000, Innogenetics, Belgium), glial fibrillary acidic protein [GFAP, rabbit polyclonal antibody (PAB), 1:2,000, Dako, Denmark]; ubiquitin (rabbit PAB, 1:5,000, Dako, Denmark), α-synuclein (LB-509, mouse MAB, 1:250, Zymed, CA, USA), and TDP-43 (rabbit PAB, 1:4,000, Proteintech, Inc., IL, USA) and rabbit polyclonal C-terminal (C-t, 1:30,000) and N-terminal TDP-43 (N-t, 1:30,000, both kindly supplied by Dr Virginia Lee, University of Pennsylvania, PA, USA) [33].

Genetic analysis

High-molecular weight DNA was extracted from whole blood, serum, or brain tissue according to standard procedures. DNA from serum was whole-genome amplified using the REPLI-g® Midi Kit (Qiagen Inc., Valencia, CA, USA) prior to genetic analysis. DNA from a single affected individual from each family was used for sequencing of *TARDBP*. All exons and the intron-exon boundaries as well as the complete 3'UTR of the *TARDBP* gene

were amplified using gene-specific intronic primers. Direct sequencing of the amplified fragments was performed using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Wellesley, MA, USA) and standard protocols. For most of the fragments, the primers used for sequencing were the same as those used for PCR amplification (primer sequences available on request). Reactions were run on an ABI 3130 DNA sequencer (AME Bioscience A/S, Torød, Norway) and mutation analysis was performed using Sequencher software v4.6 (Gene Codes Corporation, Ann Arbor, MI, USA). Where possible, sequence variants were tested for segregation with the disease and screened in a set of 982 (1,964 alleles) unrelated ethnically matched controls. Genotyping of the *APOE* ϵ polymorphism was performed using PCR amplification of a 244 base pair fragment followed by digestion with the restriction enzyme *Hha*I, as described by Hixson and Vernier [31].

RNA isolation and *TARDBP* expression analyses

RNA was isolated from a fragment of frozen frontal lobe using (RNeasy, Qiagen Inc., Valencia, CA, USA) and cDNA synthesis (High-Capacity cDNA Archive Kit, Applied Biosystems, Foster City, CA, USA). There was insufficient material for protein chemistry. The quality of mRNA was determined by UV spectrophotometer absorbance; the A260:A280 ratio was determined for each sample and all cases with a ratio of 1.95–2.00 were analyzed. Complementary DNA was assessed for genomic DNA contamination by PCR amplification with intronic-specific primers. Real-time PCR was performed using a 20 μ L total reaction with SYBR Green I 2 $\times$ master mix (Applied Biosystems, Foster City, CA, USA) with 400 nM gene-specific primers and 2 μ L of a 1:20 and 1:40 dilution of cDNA template replicated three times for each dilution. An ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) was used for PCR amplification. Efficiency of each PCR reaction was determined by six serial dilutions undertaken in duplicate and repeated three times ($n = 40$ controls, with 3 separate extractions from the one case studied). Complementary DNA (cDNA) and each mRNA expression ratio were based on normalization with glyceraldehyde-3-phosphate dehydrogenase (GAP-DH) cDNA. We calculated the variant: non-variant allele expression ratio by allele-specific real-time PCR. First, we calculated the efficiency of the allele-specific TaqMan (Applied Biosystems, Foster City, CA, USA) probes (sequence available upon requests) with serial dilutions of a heterozygous cDNA for the variant. The TaqMan real-time PCR measures accumulation of a product via the fluorophore during the exponential stages of the PCR, rather than at the end point as in conventional PCR. Secondly, we confirmed that the technique was able to quantify the amount of the variant allele in relation to the normal allele by generating a standard curve mixing the variant genomic DNA with the normal genomic DNA at different ratios, as previously described [22]. Finally, the variant: non-variant allele expression ratio was calculated by comparing the Ct value for the FAM probe (variant allele, A) with that of the VIC probe (normal allele, G) using heterozygous genomic DNA as a reference.

Statistics

Analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 14.0, Chicago, IL, USA). Data were analyzed using ANOVA and χ^2 tests; data are expressed as the mean $\pm$ standard deviation.

Results

TARDBP mutation analysis

Sequencing analysis of *TARDBP* previously led to the identification of a novel guanine to adenine variant in the 3' segment of the untranslated region (3'-UTR) adjacent to exon 6 (NM_007375.3, position 2076G>A) of *TARDBP* (Fig. 1b) in two genealogically unrelated

families [26]. The guanine nucleotide is highly conserved through the evolutionary spectrum in the 3'-UTR from *Homo sapiens* to *Gallus gallus* (Fig. 1c). The genomic sequence is also conserved in the following species: *Bos Taurus*, *Canis familiaris*, *Cavia porcellus*, *Dipodomys ordii*, *Equus caballus*, *Erinaceus europaeus*, *Felis catus*, *Gorilla gorilla*, *Loxodonta africana*, *Microcebus murinus*, *Monodelphis domestica*, *Myotis lucifugus*, *Otolemur garnettii*, *Pteropus vampyrus*, *Spermophilus tridecemlineatus*, *Tarsius syrichta*, *Tursiops truncatus*, and *Vicugna pacos* (<http://www.ensembl.org>), supporting its likely functional importance. The 3'-UTR variant segregated with both affected members of a family with clinical heterogeneity including both bvFTD and ALS/MND. Also, this variant was present in an unrelated familial ALS patient; no neuropathology was available for that case. The variant found in these two families was absent from a large series of ethnically matched elderly controls ($n = 974$ cases; $n = 1,948$ alleles; frequency = 0.00051). With 1,948 chromosomes screened and taking a hypothetical population frequency of 1%, the power to detect a variant was >0.999. The proband's *APOE* genotype was $\epsilon 2:\epsilon 3$.

TARDBP 3'-UTR functional analysis

We studied the allele-specific gene expression in 3'-UTR variant cases and in four non-carriers. Genomic DNA from a variant carrier was used as a reference. Our analyses indicated that the variant allele was expressed at significantly higher levels (22%, $P < 0.05$) than the normal allele. One limitation of this technique is that this increase in allele-specific gene expression may be caused by another variant on the same chromosome as this variant. Sequencing of the entire 3'UTR region revealed that no other unique variants were discovered. To determine the effect of the 3'-UTR variant on total *TARDBP* expression levels, 40 age matched, cognitively and neurologically normal control subjects were compared with the expression levels of the proband calculated from three independent experiments. We observed a twofold increase in total expression of *TARDBP* normalized to *GAPDH* in the 3'-UTR variant carrier (0.61 ± 0.05) compared with 40 normal controls (0.30 ± 0.07 ; $P < 0.001$; Fig. 2, upper panel). To determine the specificity of the allele ratio change in the variant, we further evaluated expression levels in the following cases: FTLT with VCP mutation ($n = 1$), sporadic FTLT-TDP ($n = 2$), and familial MND with *TARDBP* mutation ($n = 1$), and observed a similar twofold increase in *TARDBP* expression (Table 1; Fig. 2, lower panel).

Micro-RNA (miRNA) binding searches of *TARDBP* showed binding of rat (rno-miR-330) miRNA directly to the 3'UTR variant region with no strong affinity for the human miR-330 (Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, <http://www.cbio.mskcc.org/cgi-bin/mirnaviewer/mirnaviewer.pl>) [63]. The nearest human miRNA-binding site (hsa-miR-130p) was located 26 base pairs upstream of the 3'UTR variant we describe here (Wellcome Trust Genome Sanger Institute, Hinxton, Cambridge, UK, <http://microrna.sanger.ac.uk/index.shtml>). The increase in the total *TARDBP* mRNA expression level and allele-specific expression levels could not be related to any known interaction with human micro-RNA-binding sites in silico.

Clinical phenotype

The proband, case 5 in our previously published study [26], was a 70-year-old Caucasian woman and was first evaluated at the Northwestern Cognitive Neurology and Alzheimer Disease Center at the age of 66 years, 4 years prior to her death in 1984, and after a 2-year history of cognitive changes, consisting of obsessive compulsive tendencies and other unusual behaviors. In contrast, other members of this family had an ALS/MND phenotype: a brother had died 6 years prior to the proband's death with lower MND, the mother died of lower MND, and the father died of ALS. A neurologically and cognitively unimpaired sister died of Hodgkin's lymphoma. The proband was college educated and had a Wechsler Adult

Intelligence Scale-Revised (WAIS-R) Full Scale IQ of 85, which was low given her prior level of functioning as a bank teller. The Wechsler Memory Scale-Revised (WMS-R) General Memory Index (comparable to IQ score) was 90, within the normal range, but, again, somewhat lower than expected. The Attention/Concentration Index was 77, suggesting that attention deficits were more prominent than memory deficits. The Trail-Making Test, a neuropsychological test of visual attention and task switching, showed impairment. The differential diagnosis was consistent with a degenerative brain disease (high probability) and bipolar disorder (low probability). Neurologic sensory-motor examination at the time was considered normal. However, she had a left Babinski sign and dystonic posturing of the arms during stressed gait, with the left worse than the right. Medications at the time were Prempro[®], a combination of estrogens and a progestin to manage moderate to severe symptoms of menopause, and multivitamins with zinc. Over time, verbal fluency became reduced and she was noted to be apathetic and socially inappropriate. In the final evaluation, 8 months prior to death, she had pulmonary problems and had added aspirin, ginkgo, and vitamin E to her medication regimen. The Mini-Mental State Examination (MMSE) score was 12 out of a possible 30, indicating moderate to severe cognitive impairment, and all test scores had declined significantly with the exception of constructional apraxia; the clinical profile was that of a progressive compartmental/executive dysfunction, or frontotemporal dementia according to the criteria of Neary et al. [49]. In summary, the proband died after a 6-year course of disease. The clinical diagnosis at expiration was possible Alzheimer's disease versus bvFTD and the clinical profile was that of progressive compartmental executive dysfunction.

Neuropathology

In the proband, case 5 of our previously published study [26], with bvFTD and no clinical evidence of MND, a brain-only autopsy was performed. The brain weighed 1,150 g (normal range 1,250–1,400 g). External examination revealed mild cerebrovascular atherosclerosis. There was moderate frontal and temporal and mild parietal lobar atrophy (Fig. 3a). Coronal sections of the cerebral hemispheres revealed moderate hippocampal and mild caudate nucleus atrophy, and moderate dilatation of the lateral ventricles (Fig. 3b). Transverse sections of the brain stem revealed mild pallor of the locus coeruleus and substantia nigra (Fig. 3c). The cerebellum was unremarkable. Microscopic examination was significant for patchy mild superficial microvacuolation and gliosis in the frontal and temporal cortices (Fig. 4a), and severe neuronal loss and gliosis in the subiculum and hippocampal CA1 subfield (Fig. 4b), consistent with hippocampal sclerosis. There was a mild neuronal loss and gliosis in the substantia nigra and locus coeruleus. Although the spinal cord was not available, there was no evidence of motor neuron loss from the motor nuclei of the brain stem. However, histologic examination of the hypoglossal nucleus revealed some shrunken or chromatolytic neurons and Bunina bodies were observed in some remaining lower motor neurons (Fig. 4c). Ubiquitin and TDP-43 immunohistochemistry (IHC) revealed the characteristic stigmata of FTLD-TDP: neuronal cytoplasmic inclusions (NCIs) and dystrophic neurites (DNs) in the frontal and temporal neocortex and numerous NCIs in hippocampal dentate granule neurons (Fig. 5a, b). NCIs were present in both superficial (Fig. 5c) and deep (Fig. 5d) cortical layers. No neuronal intranuclear inclusions (NII) were seen. There were also NCIs in the caudate nucleus and putamen: sparse with ubiquitin IHC and numerous with TDP-43 IHC (Fig. 5e, f), and dystrophic neurites (DNs) were sparse. The density and distribution of these inclusions is consistent with FTLD-TDP type 2 [17]. There were no ubiquitin- or TDP-43-immunoreactive 'skein-like' or 'globular' NCIs or Lewy-like bodies in brain stem nuclei, including the hypoglossal neurons, as are frequently present in the motor neurons of ALS. Phosphorylation-dependent anti-tau antibodies labeled pre-tangles and neurofibrillary tangles in layer II of the entorhinal cortex and rare neurofibrillary tangles and dystrophic neurites in the hippocampus, consistent with Braak

and Braak neurofibrillary tangle stage II [11,12]. There were no cortical A β or neuritic plaques as detected by thioflavin S. In the nosology of Alzheimer's disease (AD), the absence of diffuse or neuritic plaques and modest numbers of tangles excludes a neuropathologic diagnosis of AD by Khachaturian [38], CERAD [44] and NIA-Reagan Institute criteria [1].

The brother's 1993 autopsy report from the University of Chicago Hospitals was reviewed. The brain weight was 1,430 g and there was no external gross atrophy. Microscopic findings were compatible with ALS. Selected paraffin blocks (motor cortex, hippocampus, basal ganglia, midbrain, medulla, and cervical and thoracic spinal cord) from the brother's autopsy were generously loaned to us by Dr. Robert Wollman from the University of Chicago Hospitals. Sections were evaluated with hematoxylin and eosin and immunostains for ubiquitin and TDP-43. There was mild superficial microvacuolation and gliosis in the motor cortex, moderate cell loss in the substantia nigra, severe neuronal loss in the hypoglossal nucleus, moderate to severe neuronal loss in anterior horn neurons, and severe ventral nerve root atrophy. There were sparse Bunina bodies (Fig. 6a), skein-like inclusions (Fig. 6b), and Lewy-like bodies (Fig. 6c) in hypoglossal and anterior horn neurons and large TDP-43-positive (Fig. 6d) alpha-synuclein-negative (not shown) Lewy-like bodies in substantia nigra neurons. TDP-43-positive glial inclusions were identified in the anterior horns (Fig. 6e). Interestingly, sparse to moderate cytoplasmic inclusions in the hippocampal dentate gyrus, comparable to those seen in the proband with bvFTD, were also found (Fig. 7). As expected, in both the proband and her brother, dentate gyrus inclusions were labeled with TDP-43 C-t but not N-t antibodies while lower motor neuron inclusions were labeled with both C-t and N-t antibodies (Fig. 8).

Discussion

In this study we describe the neuropathology and functional data of one case with bvFTD, but not ALS/MND, and FTLT-TDP with a variant in the highly conserved 3'-UTR of *TARDBP*. To our knowledge, this is the first report of a variant in the 3'UTR with bvFTD with pathologically confirmed FTLT-TDP. This variant was found in two genealogically different families, one with both bvFTD and MND phenotypes and a separate family with MND, but was absent in controls. The functional significance of this variant was assessed through allele-specific expression and the variant allele was significantly increased in comparison to the normal allele, and there was an overall increase in the total amount of TDP-43 mRNA. Recently, adeno-associated viral (AAV) overexpression of TDP-43 in the brain of rats was reported to recapitulate some features of FTLT and MND [57]. Further evidence of *TARDBP* overexpression was reported in yeast where the formation of cytoplasmic inclusions was reminiscent of inclusion formation in human disease [36]. These data suggest that the overexpression of TDP-43 alone may play a pivotal role in the pathogenesis of TDP-43 proteinopathy. To date, most of the reported mutations have been in exon 6 in FALS, but a number of apparently sporadic ALS cases with *TARDBP* variants have also been described [26,37,39,55,56,58]. As in other autosomal dominant neurodegenerative diseases, such as FTLT with microtubule-associated protein tau (MAPT) mutation, a single mutation within one family may generate a variable age at onset and clinically and neuropathologically heterogeneous phenotypes involving either cognitive or motor systems, or combinations of both, within a single family [35,40]. Notably, although the proband (case 5 [26]) presented with bvFTD and the proband's brother presented with MND/ALS, the FTLT-TDP and ALS pathology of these two individuals overlapped. The identification of a genetic variant outside the translated region, as reported here, indicates that there may be other functionally significant regions that may alter gene expression and/or splicing and produce a more varied clinical and pathological phenotype than those recently reported in FALS [26,37,39,55,56,58]. Further studies of the non-coding regions of

TARDBP in other families, are required to determine the frequency of these variants and to determine potential genetic and clinicopathologic correlations. Mutant *TARDBP* may compromise the physiologic role of TDP-43 and lead directly or indirectly to aggregation, hyperphosphorylation, and C-terminal cleavage, the signature pathology of the TDP-43 proteinopathies [3,30,34,51].

In this and previous studies, the variability in labeling of inclusions with N-terminal- and C-terminal-specific TDP-43 antibodies is evidence that there may be region-specific pathways of TDP-43 processing, in that TDP-43-positive inclusions in neocortex and hippocampus are enriched in C-terminal fragments, a disease-specific feature of FTLTDP, while spinal cord inclusions appear to be composed of full-length TDP-43 [33]. Interestingly, glia in the hypoglossal nucleus of the cases with the 3'UTR variant appeared to label with the N-t-specific antibody but only faintly with C-t-specific anti-TDP-43 antibodies. In the report by Igaz et al. [33], glial inclusions were reported to contain both N- and C-terminal epitopes of TDP-43, and the differences in staining intensity and distribution in these two studies may be caused by differences in immunohistochemical techniques.

As anti-TDP-43 antibodies become more widely used, an increasing number of diseases are being recognized with TDP-43 proteinopathy co-morbidity, including: Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease, corticobasal degeneration, argyrophilic grain disease, hippocampal sclerosis, ALS-parkinsonism dementia complex of Guam, Perry syndrome, and as pathological aggregates in inclusion body myopathy [2,23,25,29,48,61,62]. The contribution, if any, of *TARDBP* variants to the pathogenesis of these other disorders is unknown.

FTLD with TDP-43 proteinopathy is now recognized as the most frequent single pathological entity found in autopsy series of FTLT cases [9,17]. Although the genetic cause is unknown in most cases, the most frequent cause of familial TDP-43 proteinopathy is FTLT with *GRN* mutation [6,7,19,21,24,46,47,53]. A rare cause is FTLT with *VCP* mutation [27,50,60] and other familial cases of FTLT-MND have been linked to chromosome 9 with putative variants in *ITF74* and *UBAP1* [45,54]. Immuno-histochemical and morphological studies of the subtype, density, and distribution of ubiquitin- and TDP-43-immunoreactive inclusions reveal a close association between genotype and neuropathological phenotype [18]. In one classification system, FTLT-TDP type 1 has been most closely associated with sporadic cases while FTLT with *GRN* mutation correlates most closely with type 3, and FTLT with *VCP* mutation, which has predominantly NIIs, is classified as type 4, and familial FTLT with and without MND has predominantly NCIs and DNs is type 2 [17,18] and is most consistent with the pattern and distribution of pathology seen in the proband with the *TARDBP* 3'-UTR variant.

TDP-43 regulates gene expression by several mechanisms including transcription and splicing through binding to RNA and DNA [4,5]. In vitro studies indicate that the deletion of the C-terminal domain of TDP-43 results in altered solubility, cellular mislocalization, and the formation of nuclear and cytoplasmic aggregates reminiscent of the pathology of TDP-43 proteinopathies [4,5]. The increase in allele-specific amplification, found in the proband (case 5 [26]), suggests that the variant may contribute to neurodegeneration by dysregulation of *TARDBP* gene expression via a mechanism yet to be elucidated. However, we cannot completely exclude the possibility that the variant is a benign polymorphism and further in vitro and genetic analysis of other families are needed to determine the frequency of this variant and its role in modifying the regulation of *TARDBP*. Therefore, *TARDBP* expression may contribute to clinically and neuropathologically heterogeneous phenotypes linked by a common molecular pathology, TDP-43 proteinopathy.

The clinical, neuropathologic, genetic, and functional data described here have important implications for a spectrum of diseases including ALS/MND, FTLD-MND, and FTLD-TDP which are linked by a common molecular pathology. The discovery of a variant in the *TARDBP* 3'-UTR in a patient with clinical bvFTD and pathologic confirmation of FTLD-TDP in a family with dominantly inherited bvFTD and MND may prove to provide some support for a direct link between TDP-43 function and clinical and neuropathologic heterogeneity. The combination of additional genetic and/or environmental factors contributing to this heterogeneity remains to be elucidated. The identification of a novel 3'-UTR variant implicates potentially different pathogenetic mechanisms than those described in exon 6 in FALS. In addition, this novel genetic locus within the *TARDBP* gene may generate novel targets for therapeutic intervention.

Acknowledgments

Support for this work was provided by grants from the NIH (National Institute on Aging, P30-AG13854, E.H.B., N.J., S.W., M.M.; P50-AG05681, P01-AG03991, U01-AG16976, P30-AG13854, and P30-NS057105, P50 AG16574; J.C.M., A.M.G., N.J.C., M.A.G., R.R.), the Hope Center for Neurological Disorders, the Buchanan Fund, the Barnes-Jewish Hospital Foundation, the Pacific Alzheimer's Disease Research Foundation (#C06-01) and the Charles Knight Fund. We thank the clinical, genetic, pathology, and technical staff of the collaborating centers for making information and DNA/tissue samples available for this study and we thank the families of patients whose generosity made this research possible.

References

1. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging*. 1997; 18(Suppl):S1–S2. [PubMed: 9330978]
2. Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, Graff-Radford NR, Hutton ML, Dickson DW. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol*. 2007; 61:435–445. [PubMed: 17469117]
3. 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; 351:602–611. [PubMed: 17084815]
4. Ayala YM, Misteli T, Baralle FE. TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. *Proc Natl Acad Sci USA*. 2008; 105:3785–3789. [PubMed: 18305152]
5. Ayala YM, Zago P, D'Ambrogio A, Xu YF, Petrucelli L, Buratti E, Baralle FE. Structural determinants of the cellular localization and shuttling of TDP-43. *J Cell Sci*. 2008; 121:3778–3785. [PubMed: 18957508]
6. 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; 442:916–919. [PubMed: 16862116]
7. Behrens MI, Mukherjee O, Tu PH, Liscic RM, Grinberg LT, Carter D, Paulsmeyer K, Taylor-Reinwald L, Gitcho M, Norton JB, Chakraverty S, Goate AM, Morris JC, Cairns NJ. Neuropathologic heterogeneity in HDDD1: a familial frontotemporal lobar degeneration with ubiquitin-positive inclusions and progranulin mutation. *Alzheimer Dis Assoc Disord*. 2007; 21:1–7. [PubMed: 17334266]
8. 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. TARDBP mutations in motoneuron disease with frontotemporal lobar degeneration. *Ann Neurol*. 2009; 65:470–4739. [PubMed: 19350673]

9. Bigio EH. Update on recent molecular and genetic advances in frontotemporal lobar degeneration. *J Neuropathol Exp Neurol*. 2008; 67:635–648. [PubMed: 18596549]
10. Bose JK, Wang IF, Hung L, Tarn WY, Shen CK. TDP-43 overexpression enhances exon 7 inclusion during the survival of motor neuron pre-mRNA splicing. *J Biol Chem*. 2008; 283:28852–28859. [PubMed: 18703504]
11. Braak H, Alafuzoff I, Arzberger T, Kretschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol*. 2006; 112:389–404. [PubMed: 16906426]
12. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. 1991; 82:239–259. [PubMed: 1759558]
13. 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; 276:36337–36343. [PubMed: 11470789]
14. Buratti E, Brindisi A, Giombi M, Tisminetzky S, Ayala YM, Baralle FE. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: an important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. *J Biol Chem*. 2005; 280:37572–37584. [PubMed: 16157593]
15. Buratti E, Brindisi A, Pagani F, Baralle FE. Nuclear factor TDP-43 binds to the polymorphic TG repeats in CFTR intron 8 and causes skipping of exon 9: a functional link with disease penetrance. *Am J Hum Genet*. 2004; 74:1322–1325. [PubMed: 15195661]
16. Buratti E, Dork 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; 20:1774–1784. [PubMed: 11285240]
17. Cairns NJ, Bigio EH, Mackenzie IR, Neumann M, Lee VM, Hatanpaa KJ, White CL III, Schneider JA, Grinberg LT, Halliday G, Duyckaerts C, Lowe JS, Holm IE, Tolnay M, Okamoto K, Yokoo H, Murayama S, Woulfe J, Munoz DG, Dickson DW, Ince PG, Trojanowski JQ, Mann DM. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. *Acta Neuropathol*. 2007; 114:5–22. [PubMed: 17579875]
18. Cairns NJ, Neumann M, Bigio EH, Holm IE, Troost D, Hatanpaa KJ, Foong C, White CL III, Schneider JA, Kretschmar HA, Carter D, Taylor-Reinwald L, Paulsmeier K, Strider J, Gitcho M, Goate AM, Morris JC, Mishra M, Kwong LK, Stieber A, Xu Y, Forman MS, Trojanowski JQ, Lee VM, Mackenzie IR. TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol*. 2007; 171:227–240. [PubMed: 17591968]
19. Cruts M, Gijselinck I, van der ZJ, Engelborghs S, Wils H, Pirici D, Rademakers R, Vandenberghe R, Dermaut B, Martin JJ, van Duijn C, Peeters K, Sciot R, Santens P, De Pooter T, Mattheijssens M, van den BM, Cuijt I, Vennekens K, De Deyn PP, Kumar-Singh S, Van Broeckhoven C. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*. 2006; 442:920–924. [PubMed: 16862115]
20. Daoud H, Valdmanis PN, Kabashi E, Dion P, Dupre N, Camu W, Meininger V, Rouleau GA. Contribution of TARDBP mutations to sporadic amyotrophic lateral sclerosis. *J Med Genet*. 2008; 124:649–658.
21. Davion S, Johnson N, Weintraub S, Mesulam MM, Engberg A, Mishra M, Baker M, Adamson J, Hutton M, Rademakers R, Bigio EH. Clinicopathologic correlation in PGRN mutations. *Neurology*. 2007; 69:1113–1121. [PubMed: 17522386]
22. De Luca V, Strauss J, Sernalul M, Huang S, Li PP, Warsh JJ, Kennedy JL, Wong AH. Analysis of BDNF Val66Met allele-specific mRNA levels in bipolar disorder. *Neurosci Lett*. 2008; 441:229–232. [PubMed: 18606495]
23. Fujishiro H, Uchikado H, Arai T, Hasegawa M, Akiyama H, Yokota O, Tsuchiya K, Togo T, Iseki E, Hirayasu Y. Accumulation of phosphorylated TDP-43 in brains of patients with argyrophilic grain disease. *Acta Neuropathol*. 2008; 117:151–158. [PubMed: 19039597]
24. Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, Crook R, Melquist S, Kuntz K, Petersen R, Josephs K, Pickering-Brown SM, Graff-Radford N, Uitti R, Dickson D, Wszolek Z, Gonzalez J, Beach TG, Bigio E, Johnson N, Weintraub S, Mesulam M, White CL III, Woodruff B, Caselli R, Hsiung GY, Feldman H, Knopman D, Hutton M, Rademakers R. Mutations in

- progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. *Hum Mol Genet.* 2006; 15:2988–3001. [PubMed: 16950801]
25. Geser F, Winton MJ, Kwong LK, Xu Y, Xie SX, Igaz LM, Garruto RM, Perl DP, Galasko D, Lee VM, Trojanowski JQ. Pathological TDP-43 in parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. *Acta Neuropathol.* 2008; 115:133–145. [PubMed: 17713769]
 26. Gitcho MA, Baloh RH, Chakraverty S, Mayo K, Norton JB, Levitch D, Hatanpaa KJ, White CL III, 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; 63:535–538. [PubMed: 18288693]
 27. Gitcho MA, Strider J, Carter D, Taylor-Reinwald L, Forman MS, Goate AM, Cairns NJ. VCP mutations causing frontotemporal lobar degeneration disrupt localization of TDP-43 and induce cell death. *J Biol Chem.* 2009; 284:12384–12398. [PubMed: 19237541]
 28. Hahnen E, Schonling J, Rudnik-Schoneborn S, Zerres K, Wirth B. Hybrid survival motor neuron genes in patients with autosomal recessive spinal muscular atrophy: new insights into molecular mechanisms responsible for the disease. *Am J Hum Genet.* 1996; 59:1057–1065. [PubMed: 8900234]
 29. Hasegawa M, Arai T, Akiyama H, Nonaka T, Mori H, Hashimoto T, Yamazaki M, Oyanagi K. TDP-43 is deposited in the Guam parkinsonism-dementia complex brains. *Brain.* 2007; 130:1386–1394. [PubMed: 17439983]
 30. Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle F, Morita M, Nakano I, Oda T, Tsuchiya K, Akiyama H. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol.* 2008; 64:60–70. [PubMed: 18546284]
 31. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res.* 1990; 31:545–548. [PubMed: 2341813]
 32. Hu WT, Josephs KA, Knopman DS, Boeve BF, Dickson DW, Petersen RC, Parisi JE. Temporal lobar predominance of TDP-43 neuronal cytoplasmic inclusions in Alzheimer disease. *Acta Neuropathol.* 2008; 116:215–220. [PubMed: 18592255]
 33. Igaz LM, Kwong LK, Xu Y, Truax AC, Uryu K, Neumann M, Clark CM, Elman LB, Miller BL, Grossman M, McCluskey LF, Trojanowski JQ, Lee VM. Enrichment of C-terminal fragments in TAR DNA-binding protein-43 cytoplasmic inclusions in brain but not in spinal cord of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Am J Pathol.* 2008; 173:182–194. [PubMed: 18535185]
 34. Inukai Y, Nonaka T, Arai T, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle FE, Akiyama H, Hisanaga S, Hasegawa M. Abnormal phosphorylation of Ser409/410 of TDP-43 in FTL-D-U and ALS. *FEBS Lett.* 2008; 582:2899–2904. [PubMed: 18656473]
 35. Janssen JC, Warrington EK, Morris HR, Lantos P, Brown J, Revesz T, Wood N, Khan MN, Cipolotti L, Fox NC, Rossor MN. Clinical features of frontotemporal dementia due to the intronic tau 10(+16) mutation. *Neurology.* 2002; 58:1161–1168. [PubMed: 11971081]
 36. Johnson BS, Snead D, Lee JJ, McCaffery JM, Shorter J, Gitler AD. TDP-43 is intrinsically aggregation-prone and ALS-linked mutations accelerate aggregation and increase toxicity. *Proc Natl Acad Sci USA.* 2008; 105:6439–6444. [PubMed: 18434538]
 37. Kabashi E, Valdmanis PN, Dion P, Spiegelman D, McConkey BJ, Vande VC, Bouchard JP, Lacomblez L, Pochigaeva K, Salachas F, Pradat PF, Camu W, Meininger V, Dupre N, Rouleau GA. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet.* 2008; 40:572–574. [PubMed: 18372902]
 38. Khachaturian ZS. Diagnosis of Alzheimer's disease. *Arch Neurol.* 1985; 42:1097–1105. [PubMed: 2864910]
 39. Kuhnlein P, Sperfeld AD, Vanmassenhove B, Van Deerlin V, Lee VM, Trojanowski JQ, Kretzschmar HA, Ludolph AC, Neumann M. Two German kindreds with familial amyotrophic lateral sclerosis due to TARDBP mutations. *Arch Neurol.* 2008; 65:1185–1189. [PubMed: 18779421]

40. Lantos PL, Cairns NJ, Khan MN, King A, Revesz T, Janssen JC, Morris H, Rossor MN. Neuropathologic variation in frontotemporal dementia due to the intronic tau 10(+16) mutation. *Neurology*. 2002; 58:1169–1175. [PubMed: 11971082]
41. Liscic RM, Grinberg LT, Zidar J, Gitcho MA, Cairns NJ. ALS and FTLT: two faces of TDP-43 proteinopathy. *Eur J Neurol*. 2008; 15:772–780. [PubMed: 18684309]
42. Mackenzie IR, Bigio EH, Ince PG, Geser F, Neumann M, Cairns NJ, Kwong LK, Forman MS, Ravits J, Stewart H, Eisen A, McClusky L, Kretzschmar HA, Monoranu CM, Highley JR, Kirby J, Siddique T, Shaw PJ, Lee VM, Trojanowski JQ. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol*. 2007; 61:427–434. [PubMed: 17469116]
43. Mackenzie IR, 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 AJ, Kumar-Singh S, Akiyama H, Baborie A, Spina S, Dickson DW, Trojanowski JQ, Mann DM. Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. *Acta Neuropathol*. 2009; 117:15–18. [PubMed: 19015862]
44. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology*. 1991; 41:479–486. [PubMed: 2011243]
45. Momeni P, Schymick J, Jain S, Cookson MR, Cairns NJ, Greggio E, Greenway MJ, Berger S, Pickering-Brown S, Chio A, Fung HC, Holtzman DM, Huey ED, Wassermann EM, Adamson J, Hutton ML, Rogaeva E, George-Hyslop P, Rothstein JD, Hardiman O, Grafman J, Singleton A, Hardy J, Traynor BJ. Analysis of IFT74 as a candidate gene for chromosome 9p-linked ALS-FTD. *BMC Neurol*. 2006; 6:44. [PubMed: 17166276]
46. Mukherjee O, Pastor P, Cairns NJ, Chakraborty S, Kauwe JS, Shears S, Behrens MI, Budde J, Hinrichs AL, Norton J, Levitch D, Taylor-Reinwald L, Gitcho M, Tu PH, Tenenholz GL, Liscic RM, Armendariz J, Morris JC, Goate AM. HDDD2 is a familial frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusions caused by a missense mutation in the signal peptide of progranulin. *Ann Neurol*. 2006; 60:314–322. [PubMed: 16983685]
47. Mukherjee O, Wang J, Gitcho M, Chakraborty S, Taylor-Reinwald L, Shears S, Kauwe JS, Norton J, Levitch D, Bigio EH, Hatanpaa KJ, White CL, Morris JC, Cairns NJ, Goate AM. Molecular characterization of novel progranulin (GRN) mutations in frontotemporal dementia. *Hum Mutat*. 2008; 29:512–521. [PubMed: 18183624]
48. Nakashima-Yasuda H, Uryu K, Robinson J, Xie SX, Hurtig H, Duda JE, Arnold SE, Siderowf A, Grossman M, Leverenz JB, Woltjer R, Lopez OL, Hamilton R, Tsuang DW, Galasko D, Masliah E, Kaye J, Clark CM, Montine TJ, Lee VM, Trojanowski JQ. Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. *Acta Neuropathol*. 2007; 114:221–229. [PubMed: 17653732]
49. Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M, Boone K, Miller BL, Cummings J, Benson DF. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology*. 1998; 51:1546–1554. [PubMed: 9855500]
50. Neumann M, Mackenzie IR, Cairns NJ, Boyer PJ, Markesbery WR, Smith CD, Taylor JP, Kretzschmar HA, Kimonis VE, Forman MS. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *J Neuropathol Exp Neurol*. 2007; 66:152–157. [PubMed: 17279000]
51. 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. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006; 314:130–133. [PubMed: 17023659]
52. Ou SH, Wu F, Harrich D, Garcia-Martinez 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; 69:3584–3596. [PubMed: 7745706]
53. Rademakers R, Baker M, Gass J, Adamson J, Huey ED, Momeni P, Spina S, Coppola G, Karydas AM, Stewart H, Johnson N, Hsiung GY, Kelley B, Kuntz K, Steinbart E, Wood EM, Yu CE,

- Josephs K, Sorenson E, Womack KB, Weintraub S, Pickering-Brown SM, Schofield PR, Brooks WS, Van Deerlin VM, Snowden J, Clark CM, Kertesz A, Boylan K, Ghetti B, Neary D, Schellenberg GD, Beach TG, Mesulam M, Mann D, Grafman J, Mackenzie IR, Feldman H, Bird T, Petersen R, Knopman D, Boeve B, Geschwind DH, Miller B, Wszolek Z, Lippa C, Bigio EH, Dickson D, Graff-Radford N, Hutton M. Phenotypic variability associated with progranulin haploinsufficiency in patients with the common 1477C→T (Arg493X) mutation: an international initiative. *Lancet Neurol.* 2007; 6:857–868. [PubMed: 17826340]
54. Rollinson S, Rizzu P, Sikkink S, Baker M, Halliwell N, Snowden J, Traynor BJ, Ruano D, Cairns N, Rohrer JD, Mead S, Collinge J, Rossor M, Akay E, Guerreiro R, Rademakers R, Morrison KE, Pastor P, Alonso E, Martinez-Lage P, Graff-Radford N, Neary D, Heutink P, Mann DM, van Swieten J, Pickering-Brown SM. Ubiquitin associated protein 1 is a risk factor for frontotemporal lobar degeneration. *Neurobiol Aging.* 2009; 30:656–665. [PubMed: 19217189]
 55. Rutherford NJ, Zhang YJ, Baker M, Gass JM, Finch NA, Xu YF, Stewart H, Kelley BJ, Kuntz K, Crook RJ, 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. [PubMed: 18802454]
 56. 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; 319:1668–1672. [PubMed: 18309045]
 57. 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; 17:607–661. [PubMed: 19223871]
 58. 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 IL, Yang WS, Kalb R, Galasko DR, Montine TJ, Trojanowski JQ, Lee VM, Schellenberg GD, Yu CE. TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. *Lancet Neurol.* 2008; 7:409–416. [PubMed: 18396105]
 59. Wang HY, Wang IF, Bose J, Shen CK. Structural diversity and functional implications of the eukaryotic TDP gene family. *Genomics.* 2004; 83:130–139. [PubMed: 14667816]
 60. Watts GD, 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; 36:377–381. [PubMed: 15034582]
 61. Weihl CC, Temiz P, Miller SE, Watts G, Smith C, Forman M, Hanson PI, Kimonis V, Pestronk A. TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *J Neurol Neurosurg Psychiatry.* 2008; 79:1186–1189. [PubMed: 18796596]
 62. Wider C, Dickson DW, Stoessl AJ, Tsuboi Y, Chapon F, Gutmann L, Lechevalier B, Calne DB, Personett DA, Hulihan M, Kachergus J, Rademakers R, Baker MC, Grantier LL, Sujith OK, Brown L, Calne S, Farrer MJ, Wszolek ZK. Pallidonigral TDP-43 pathology in Perry syndrome. *Parkinsonism Relat Disord.* 2008; 15:281–286. [PubMed: 18723384]
 63. Xiangjun HE, Zhang Q, Liy Y, Pan X. Cloning and identification of novel microRNAs from rat hippocampus. *Acta Biochimica et Biophysica Sinica.* 2007; 39:708–714. [PubMed: 17805466]

Fig. 1. *TARD3P* 3'-UTR missense variant within a highly conserved region of affected members of a family with FTL and/or MND with TDP-43 proteinopathy. **a** Protein domain structure of TDP-43. Recognition motifs: *NE* nuclear export sequence, *NL* nuclear localization sequence, *RRM1* and *2* RNA recognition motifs 1 and 2, and *hnRNP* heterogeneous nuclear ribonucleoprotein (glycine-rich) domain. **b** *TARD3P* genomic structure (*blue* untranslated region, *red* coding region) and position of 3'-UTR variant. **c** *TARD3P* 3'-UTR is evolutionarily conserved between species (nucleotide in red is position of 3'-UTR variant). **d** Chromatogram of 3'-UTR displays a base-pair change (2,076 G>A, NM_007375.3). **e** Pedigree of family displaying heterogeneous clinical phenotypes: *ALS* ♦, *bvFTD* ♦, *MND* ♦; arrow proband. No DNA was available from either parent

Fig. 2.

TARDBP expression (*upper panel*) is increased in FTLD with *TARDBP* 3'-UTR variant in comparison to normal subjects. *TARDBP* expression (*lower panel*) is increased in FTLD with 3'UTR variant compared with other TDP-43 proteinopathies. The *values* expressed represent the means of at least three independent experiments repeated three times and each normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. To compensate for multiple comparisons, a Bonferroni correction was made with the variability expressed as the standard deviation: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$)

Fig. 3. Macroscopy of FTLD with *TARDBP* 3'-UTR variant. **a** Coronal slice showing moderate atrophy of the frontal pole. **b** Coronal slice of the frontal lobe showing moderate dilatation of the lateral ventricle. **c** There is mild pallor of the substantia nigra

Fig. 4. Microscopy of FTL D with *TARDBP* 3'-UTR variant. **a** Mild superficial microvacuolation and gliosis in superficial frontal cortex (H&E, ×100). **b** Severe neuronal loss and gliosis in the subiculum [glial fibrillary acidic protein (*GFAP*) immunohistochemistry, ×100]. **c** Hypoglossal motor neuron with a Bunina body (*arrow*) and a shrunken motor neuron (*arrowhead*) (H&E, ×400)

Fig. 5. Neuronal cytoplasmic inclusions (NCIs) in FTLD with *TARDBP* 3'-UTR variant. **a, b** Frequent NCIs are present in the hippocampal dentate gyrus (**a** ubiquitin, **b** TDP-43 immunohistochemistry, **a, b** $\times 400$). **c** Sparse granular NCIs (*arrows*) in the superficial temporal cortex (TDP-43 immunohistochemistry, $\times 400$). **d** Sparse granular NCIs (*arrows*) in the pyramidal neurons of cortical layer 3 (TDP-43 immunohistochemistry, $\times 400$). **e** Frequent granular NCIs in the putamen (TDP-43 immunohistochemistry, $\times 400$). **f** Moderate DN and sparse NCIs (*inset*) in the putamen (ubiquitin immunohistochemistry, $\times 400$, *inset* $\times 600$)

Fig. 6. Inclusions in the motor nuclei of the brain stem and spinal cord in FTLD with *TARDBP* 3'-UTR variant. **a** A Bunina body (*arrow*) in a cervical anterior horn neuron (H&E, $\times 600$) and **b** a delicate single neuronal cytoplasmic skein (**b** TDP-43 immunohistochemistry, $\times 600$). **c** Lewy body-like inclusions in neurons of the hypoglossal nucleus (TDP-43 immunohistochemistry, $\times 400$). **d** Lewy body-like inclusions in neurons of the substantia nigra (TDP-43 immunohistochemistry, $\times 400$). **e** A glial inclusion in the cervical cord (TDP-43 immunohistochemistry, $\times 600$)

Fig. 7.
Sparse to moderate numbers of NCIs (*arrows*) in the dentate gyrus (TDP-43 immunohistochemistry, $\times 400$)

Fig. 8. C-terminal specific anti-TDP-43 antibodies preferentially label epitopes within neuronal NCIs of FTLD with *TARDBP* 3'-UTR variant. **a, b** NCI s in the dentate gyrus of the proband. **a** C-t-specific and **b** N-t-specific TDP-43 immunohistochemistry. **a** The majority of NCIs are C-t-positive. **b** A few NCIs are only weakly labeled by N-t-specific anti-TDP-43 antibodies. The nuclei are labeled by N-t-specific anti-TDP-43 antibodies, but not by the 'disease-specific' anti-C-t antibodies. Some neurons with weak N-t labeling of NCIs have N-t-negative nuclei. (C-t- and N-t-specific TDP-43 immunohistochemistry, $\times 600$). NCIs in the dentate gyrus of the proband's brother show **(c)** similar C-terminal-specific labeling and **(d)** only weak N-terminal-specific labeling as in the proband **(a, b)**. There are sparse C-t-positive NCIs, but not N-t-positive NCIs; some nuclei, however, are N-t-negative (C-t- and N-t-specific TDP-43 immunohistochemistry, $\times 600$). Neuronal inclusion in the hypoglossal nuclei of the proband **(e)** and proband's brother **(f)**. In both **(e)** and **(f)**, C-t-specific TDP-43 immunohistochemistry is on the *left* and N-t-specific labeling is on the *right*. The lower motor neuron NCIs are labeled by both antibodies. In addition, glial cytoplasmic inclusions are labeled with anti-N-t TDP-43, but only very weakly with anti-C-t TDP-43 antibodies (*arrows*) (C-t-specific and N-t-specific TDP-43 immunohistochemistry; **e** $\times 600$, **f** $\times 400$)

Table 1
Spectrum of cases of TDP-43 proteinopathy used to determine allele expression

Case ID	Clinical/genetic phenotype	Mutation	Age at onset (years)	Duration (years)	Age at death (years)
1	FTLD with <i>TARDBP</i> 3'UTR variant	2,076 G>A	66	4	70
2	FTLD with <i>VCP</i> mutation	R155H	38	9	47
3	FTLD-TDP-1	Sporadic	62	11	73
4	FTLD-TDP-2	Sporadic	61	6	67
5	MND with <i>TARDBP</i> mutation	A315T	83	5	88