Published in final edited form as:
Arch Neurol. 2008 November ; 65(11): 1481–1487. doi:10.1001/archneur.65.11.1481.

TDP-43 in Cerebrospinal Fluid of Patients With Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis

Petra Steinacker, PhD, **Corinna Hendrich, MD**, **Anne D. Sperfeld, MD**, **Sarah Jesse, MD**, **Christine A. F. von Arnim, MD**, **Stefan Lehnert, PhD**, **Alice Pabst**, **Ingo Uttner, PhD**, **Hayrettin Tumani, MD**, **Virginia M.-Y. Lee, PhD**, **John Q. Trojanowski, MD, PhD**, **Hans A. Kretzschmar, MD**, **Albert Ludolph, MD**, **Manuela Neumann, MD**, and **Markus Otto, MD**

Department of Neurology, University of Ulm, Ulm (Drs Steinacker, Hendrich, Sperfeld, Jesse, von Arnim, Lehnert, Uttner, Tumani, Ludolph, and Otto and Ms Pabst), and Center for Neuropathology and Prion Research, Ludwig-Maximilians University Munich, Munich (Drs Kretzschmar and Neumann), Germany; and Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, and Alzheimer's Disease Core Center, Institute on Aging, University of Pennsylvania School of Medicine, Philadelphia (Drs Lee and Trojanowski).

Abstract

Background—Recently, TAR DNA-binding protein 43 (TDP-43) was identified as the major component of ubiquitin-positive tau-negative neuronal and glial inclusions in the most common form of frontotemporal lobar degeneration (FTLD) and in amyotrophic lateral sclerosis (ALS). It was demonstrated that different TDP-43 profiles correspond to clinical phenotypes of FTLD or ALS subgroups, and the differential diagnostic potential of TDP-43 was suggested.

Objectives—To examine TDP-43 in cerebrospinal fluid (CSF) and to analyze whether it could serve as a diagnostic marker.

Design—We characterized CSF TDP-43 by immunoblot using different TDP-43 antibodies and determined the relative TDP-43 levels in CSF samples from patients.

Setting—Academic research.

Patients—Twelve patients with FTLD, 15 patients with ALS, 9 patients with ALS plus FTLD, 3 patients with ALS plus additional signs of frontal disinhibition, and 13 control subjects.

Main Outcome Measures—Results of TDP-43 immunoblot.

Results—Polyclonal TDP-43 antibodies recognized a 45-kDa band in all analyzed samples. Two monoclonal and N-terminus—specific antibodies did not detect any specific bands, but C-terminus —specific antibodies detected a 45-kDa band and additional bands at approximately 20 kDa in all CSF samples. Relative quantification of 45-kDa bands revealed significant differences among the

Correspondence: Markus Otto, MD, Department of Neurology, University of Ulm, Steinhövelstrasse 1, 89075 Ulm, Germany (E-mail: markus.otto@uni-ulm.de)..

Author Contributions: *Study concept and design:* Steinacker, Jesse, Lehnert, Neumann, and Otto. *Acquisition of data:* Steinacker, Hendrich, Sperfeld, von Arnim, Pabst, Uttner, Tumani, Ludolph, and Otto. *Analysis and interpretation of data:* Steinacker, Lee, Trojanowski, Kretzschmar, Neumann, and Otto. *Drafting of the manuscript:* Steinacker and Otto. Critical revision of the manuscript for important intellectual content: Steinacker, Hendrich, Sperfeld, Jesse, von Arnim, Lehnert, Pabst, Uttner, Tumani, Lee, Trojanowski, Kretzschmar, Ludolph, Neumann, and Otto. *Statistical analysis:* Steinacker and Otto. *Obtained funding:* Steinacker and Otto. *Administrative, technical, and material support:* Steinacker, Hendrich, Sperfeld, von Arnim, Lehnert, Pabst, Tumani, Kretzschmar, Ludolph, Neumann, and Otto. *Study supervision:* Steinacker, Sperfeld, Uttner, Lee, Trojanowski, Ludolph, and Otto.

Financial Disclosure: None reported.

diagnostic groups (*P*=.046). Specifically, patients with ALS (*P*=.03) and FTLD (*P*=.02) had higher TDP-43 levels than controls but with a prominent overlap of values.

Conclusion—Although there is no evidence of pathologically altered TDP-43 proteins in CSF, TDP-43 levels in CSF might aid in characterizing subgroups of patients across the ALS and FTLD disease spectrum.

Frontotemporal lobar degeneration (FTLD) is the second most common cause of dementia affecting individuals younger than 65 years.[1] On a cellular pathologic level, most FTLD cases are characterized by the presence of ubiquitin-positive inclusions. Because of the nature of aggregated constituents of these inclusions, for which key roles in pathologic mechanisms are proposed, FTLD was categorized into 2 major groups with and without tau. In the FTLD-tau group, filamentous tau proteins form the disease inclusions.[2] Recently, pathologically phosphorylated and ubiquitinated TAR DNA-binding protein 43 (TDP-43) was identified as a major pathologic protein of sporadic and familial FTLD with ubiquitin-positive tau-negative inclusions (FTLD-U),[3,4] which constitutes more than 50% of all FTLD cases.[5]

The clinical picture of patients with FTLD-tau and FTLD-U is heterogeneous. Besides behavioral and personality changes, patients manifest language disturbances in primary progressive aphasia with progressive nonfluent aphasia and semantic dementia. In the case of corticobasal degeneration and progressive supranuclear palsy, behavioral abnormalities are accompanied by extrapyramidal features.[1] Frontotemporal lobar degeneration can be associated with neurodegeneration of motor neurons in motor cortex, brainstem, and spinal cord, leading to a syndrome with features of amyotrophic lateral sclerosis (ALS) and FTLD.[6] In ALS, the most common type of motor neuron disorder (MND), upper and lower motor neurons are affected, and most patients die of respiratory failure on average about 3 years after symptom onset.[7,8] Furthermore, approximately 15% of patients with ALS develop dementia categorized as FTLD.[9] TDP-43 is not only the main constituent of inclusions in FTLD-U with and without ALS but is also present in sporadic ALS, ALS with dementia, and *SOD1*-negative ALS.[3,10,11] This observation led to the hypothesis that TDP-43 is a common pathologic substrate in these diseases, implicating similar pathophysiologic mechanisms, despite significant clinical, genetic, and neuropathologic heterogeneity of FTLD-U and ALS.[3,12]

So far (to our knowledge), no specific laboratory marker exists for disease progression or for differential diagnostic use in ALS or FTLD. Because pathologic changes in the brain and spinal cord can be reflected by altered levels of proteins or other analytes in cerebrospinal fluid (CSF), we analyzed CSF from patients having FTLD with and without ALS, from patients having ALS with and without FTLD or signs of frontal disinhibition, and from control subjects to determine if TDP-43 could be detected in CSF and if assaying CSF TDP-43 could be used as a biomarker for the diagnosis, staging, or care of patients with FTLD-U or ALS.

METHODS

DESIGN

All 52 CSF samples analyzed in this study were obtained from patients attending the general outpatient clinic, the outpatient memory clinic, or the outpatient clinic for MND (Department of Neurology, University of Ulm, Ulm, Germany) from January 2006 to December 2007. Collection and analysis of CSF samples were approved by the ethics committee. Routine CSF data such as albumin concentration and IgG concentration were available for all samples. All individuals, or their relative in the case of patients with dementia, gave written informed consent to their participation in the study and underwent clinical, neurologic, and neuroradiologic examinations and a short neuropsychological screening, including the Mini-Mental State Examination[13] to investigate global cognitive functioning. If deterioration had

been suggested, a detailed psychometric test battery covering executive functions, memory, constructional abilities, premorbid verbal intelligence, and depression[14] was administered to assess more specifically for cognitive impairment and frontotemporal degeneration. The diagnoses of all patients were made in accord with the consensus criteria for FTLD[15] and on the basis of *DSM-IV* criteria and were established by neurologists (C.H., A.D.S., C.A.F.v.A, A.L., and M.O.) in cooperation with a neuropsychologist (I.U.), both blinded to the neurochemical outcome measures. Diagnosis of ALS was made according to the El Escorial criteria of Pradat and Bruneteau.[16]

PATIENTS WITH FTLD

The FTLD group consisted of 12 patients (7 men and 5 women). The mean (SD) age of the patients at the time of CSF sampling was 68 (8.6) years. The diagnosis of frontotemporal degeneration was made in 11 patients, and 1 patient had primary progressive aphasia subtype.

The diagnosis was supported in 11 of 12 patients by fludeoxy-glucose F 18 positron emission tomography. The results demonstrated reduced cortical glucose metabolism in the frontopolar, frontomesial, or frontotemporal region.

PATIENTS WITH ALS

The ALS group consisted of 15 patients (9 men and 6 women). The mean (SD) age was 48 (7.1) years. Eight patients were diagnosed as having laboratory-confirmed ALS, 5 patients had clinically probable ALS, 1 patient had definitive ALS with a spinal course, and 1 patient had definitive ALS with bulbar progress. Ten of 15 patients with ALS were classified as having spinal disease, 3 patients as having bulbar disease, and 2 patients as having flail arm syndrome.

PATIENTS WITH ALS PLUS ADDITIONAL SIGNS OF FRONTAL DISINHIBITION

The group of patients with ALS plus additional signs of frontal disinhibition (ALS plus DI) comprised 3 women having a mean (SD) age of 63 (14.0) years. These patients exhibited additional clinical signs of frontal disinhibition without fulfilling the diagnosis of FTLD.

PATIENTS WITH ALS PLUS FTLD

The group of patients with ALS plus FTLD comprised 9 patients (5 men and 4 women). The mean (SD) age was 63 (7.1) years. Six patients were classified as having the spinal form and 3 patients as having the bulbar form of ALS. These patients fulfilled diagnostic criteria for FTLD.[15]

CONTROL SUBJECTS

The group of controls comprised 13 patients (6 men and 7 women) with a mean (SD) age of 60 (8.0) years. The final diagnoses of the patients were as follows: complex focal seizures (n=3), polymyalgia rheumatica (n=2), polyneuropathy (n=3), carcinoma (n=1), neuropathia vestibularis (n=1), depression (n=1), migraine (n=1), and dissociative disorder (n=1).

TDP-43 IMMUNOBLOT

Cerebrospinal fluid samples were stored at -80°C until analysis, at which time they were thawed for study. Identical volumes of 50 μL of native CSF were acetone precipitated.

IgG and albumin depletion was performed according to the manufacturer's instructions (GE Healthcare, Chalfont St. Giles, United Kingdom). Purified human IgG and albumin were obtained from Sigma-Aldrich Inc (St Louis, Missouri).

Murine neuroblastoma cells were lysed in radioimmuno-precipitation assay (RIPA) buffer (150mM sodium chloride, 20mM Tris [pH 7.4], 1% NP-40, 0.05% Triton X-100, 0.5% sodium desoxycholate, and 0.5M EDTA). The homogenate served as a control and as an internal Western immunoblot standard.

Mouse whole brain was homogenized in phosphate-buffered saline (PBS) (1 mL/0.1 g of tissue) solution containing aprotinin (1 μg/mL), phenylmethylsulfonyl fluoride (0.2mM), and leupeptin (0.5 μg/mL) and was sonicated for 30 seconds. After centrifugation at 20 000*g* for 10 minutes at 4°C, the supernatant was retained, and the protein concentration was determined by bicinchoninic acid assay (BCA; Sigma-Aldrich Inc, St Louis, Missouri).

Urea fractions were prepared from frozen frontal cortex of a patient with FTLD-U. The sequential extraction protocol has been published previously.[3]

Samples were reconstituted or mixed with sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (Roti-load 1; Carl Roth GmbH, Karlsruhe, Germany) to a final concentration of 2.5% mercaptoethanol. They were boiled for 5 minutes before electrophoresis.

Proteins were separated on Laemmli gels with 12% acrylamide in the separation gel and with 4% acrylamide in the stacking gel. Electrophoresis was performed at 25 mA per gel for about 90 minutes. Proteins were transferred to polyvinylidene difluoride membranes (Millipore Corporation, Bedford, Massachusetts) by semidry blot. Membranes were blocked with PBS and 0.075% polysorbate 20 (Tween-20) containing 5% dry milk powder (Bio-Rad, Hercules, California) and were then probed with anti—TDP-43 antibodies in blocking solution. Affinity-purified polyclonal rabbit antibody was raised against amino acids 1 through 260 of recombinant TDP-43 (1:2000 and 1:10 000 to 1:1000; Proteintech Group Inc, Chicago, Illinois). Monoclonal TDP-43 antibody clone 2E2-D3 specific for amino acids 205 through 255[17] (1:1000; Abnova, Taipei City, Taiwan) was also used. Polyclonal rabbit antisera were raised against N-terminus amino acids 6 through 24 or against C-terminus amino acids 396 through 414 of TDP-43 (1:5000 for both). Immunoblots were incubated with primary antibody overnight at 4°C and for 1 hour at room temperature after 3 washing steps with peroxidase-conjugated goat antirabbit (Dianova, Hamburg, Germany) or antimouse (DAKO, Glostrup, Denmark) secondary antibody. A Western blot detection reagent (ECL Plus; GE Healthcare) was used as a substrate, and chemiluminescence was measured with a charge-coupled device camera (LAS-1000; Fujifilm, Tokyo, Japan).

CALCULATIONS AND STATISTICAL ANALYSIS

The 45-kDa TDP-43 bands from CSF detected by rabbit TDP-43 antibody (Proteintech Group Inc) were quantified in relation to a fixed amount of mouse neuroblastoma RIPA cell homogenate serving as an internal standard. Band volumes (adjusted for membrane background) were determined using commercially available software (Quantity One, Bio-Rad) and were calculated relative to the volume of the 46-kDa band present in mouse neuroblastoma cell homogenate separated in parallel on the same gel. The TDP-43 band of each patient's CSF was quantified from duplicates or triplicates run on different gels and days. Western blots in which the standard band density differed more than 30% from the mean value of standard bands determined in all immunoblots were discarded.

Analyses for significant differences in a given variable among all tested groups (Kruskal-Wallis test) or between 2 groups (Mann-Whitney test) were calculated using commercially available statistical software (SigmaStat Software; SigmaStat, ask-net AG, Karlsruhe, Germany). Correlation between variables was examined using Spearman rank correlation. $P<.05$ was considered statistically significant.

RESULTS

EVALUATION OF TDP-43 IMMUNOBLOT

Two bands were regularly detected by rabbit polyclonal anti—TDP-43 antibody in immunoblots of CSF from patients and from controls (Figure 1). The upper band migrates at 45 kDa, which is similar to the 46-kDa band detected in mouse cell lysates and higher than the 28-kDa band detected in mouse brain homogenate. In the urea fraction extracted from FTLD-U brain tissue, in addition to a 47-kDa band we found an approximately 50-kDa band of the same intensity, 2 weaker bands at 44 and approximately 60 kDa, and a high relative molecular mass smear (Figure 1B). No signals of corresponding molecular weight were detected in CSF.

The 28-kDa band present in CSF from all patients and controls quantitatively disappears by depletion of albumin and IgG from native CSF before precipitation, SDS-PAGE, and immunoblot. Intensities of the 45-kDa band in CSF were unaffected by removal of IgG and albumin. Analyzing purified human IgG and albumin (the most abundant proteins of CSF) using the polyclonal TDP-43 antibody, a band with exactly the same molecular weight of 28 kDa was detected in IgG, whereas no band was detected in purified albumin (Figure 1). Therefore, the antibody cross-reacts with the IgG light chain. Applying the commercially available monoclonal antibody raised against amino acids 1 through 260 of recombinant TDP-43 and uncharacterized with regard to the binding site, we regularly detected a 26-kDa band, a 44-kDa band, and bands of approximately 47, 50, and 60 kDa, and high relative molecular mass smear in urea fractions. There was no specific immunoreactivity in any patient or control CSF using these monoclonal antibodies in dilutions of up to 1:1000 and using chemiluminescence exposure times of up to 20 minutes (Figure 2). Several weak bands were detected in CSF using clone 2E2-D3 monoclonal TDP-43 antibody, mainly representing IgG and albumin. In some immunoblots of CSF, we found a trace band at about 42 kDa (**Figure 3**B) but no bands between 43 and 50 kDa. To exclude that the 45-kDa band in CSF detected by polyclonal antibodies is unspecific and unrelated to TDP-43, we subjected CSF immunoblots to immunodetection with polyclonal antibodies specific for the N-terminus or C-terminus of TDP-43. The 46-kDa band in murine neuroblastoma cell homogenate was strongly recognized by both antibodies. No specific bands were detected in CSF using the N-terminus—specific antiserum (Figure 3C). In contrast, C-terminus—specific antibodies bound to a protein band at 45 kDa in CSF from the same 5 samples analyzed using the N-terminus—specific antibody. In addition, protein bands at 20 kDa in 12% and at less than 20 kDa in 15% sodium dodecyl sulfate–polyacrylamide gels were detected using the C-terminus—specific antibody (Figure 3D).

TDP-43 LEVELS IN CSF OF PATIENT GROUPS

Intensities of the 45-kDa band recognized in CSF by TDP-43 polyclonal antibody were quantified in samples from 52 patients with FTLD, ALS, ALS plus FTLD, and ALS plus DI and from controls without dementia or MND. The band was stable at various preanalytic conditions. Neither storage of up to 2 days at 4°C after lumbar puncture nor 3 freeze-thaw cycles affected detected band intensities. Sample preparation affected neither size nor number of bands. Neither pretreatment of CSF with RIPA lysis buffer and subsequent acetone precipitation nor acetone precipitation of mouse cell lysates affected the molecular weight of the bands detected by polyclonal TDP-43 antibodies (data not shown).

The ALS and ALS plus FTLD groups comprised younger patients, especially compared with the FTLD group (Table 1). No correlation was noted between patient age and relative 45-kDa TDP-43 levels detected by rabbit TDP-43 antibodies in CSF immunoblots (Spearman rank correlation coefficient, -0.153; P=.28).

There was a wide variation of TDP-43 levels among our CSF samples (Table 2 and Figure 4). TDP-43 levels ranged from 7% to 164% (median, 60%) in the ALS group, 26% to 92% (median, 63%) in the FTLD group, 9% to 105% (median, 24%) in the ALS plus FTLD group, and 5% to 79% (median, 28%) in the control group. Two of 3 patients in the ALS plus DI group had low TDP-43 levels (16% and 17%), whereas 1 patient had a level of 100%. The mean (SD) values are given in Table 2. Statistical analysis revealed significant differences among all tested groups (P=.046). The ALS plus DI group was omitted from this analysis because of the small sample.

TDP-43 levels were increased in the ALS and FTLD groups compared with controls (P=.03 and P=.02, respectively). In the ALS plus FTLD group, intermediate levels of TDP-43 were found that were not statistically different from those in the ALS group (P=.15), FTLD group (P=.13), or control group (P=.89). There was no correlation with age, nor was there a relationship between TDP-43 level and the clinical phenotype of patients with ALS (bulbar vs spinal). There was no significant correlation between the CSF IgG concentration or the CSF to serum albumin ratio and the relative TDP-43 band intensity in any of the analyzed patient groups.

COMMENT

TDP-43, a nuclear protein that putatively functions in regulating transcription and alternative splicing, is a main component of inclusions in most familial and sporadic FTLD-U cases, including FTLD-U subgroups such as FTLD-U plus MND and FTLD-U manifesting clinically as semantic dementia or progressive nonfluent aphasia.[3,4,18] Moreover, examination of subgroups with MND revealed TDP-43—immunopositive neuronal and glial inclusions in sporadic ALS, ALS with dementia, and superoxide dismutase 1, soluble *SOD1*-negative familial ALS,[11] suggesting that FTLD-U and ALS represent a clini-copathologic spectrum of disorders sharing similar pathomechanisms.[3,11,19]

Demonstration of TDP-43 immunoreactivity in neuronal and glial inclusions is useful in the differential diagnosis of ALS or ALS plus FTLD from other MNDs affecting upper and lower motor neurons and confirms subclinical MND in FTLD without clinical or pathologic evidence of MND.[10] Proteins involved in patho-physiologically regulated pathways correlate with altered CSF concentrations and may be helpful in the differential diagnosis. In our immunoblot analysis applying polyclonal antibodies against TDP-43, a specific 45-kDa band was regularly present in all analyzed CSF samples. In immunoblots of urea fractions extracted from central nervous system tissue of patients with FTLD-U and ALS, Neumann et al[3] described a disease-specific biochemical profile of TDP-43 with bands of 24 and of 26 kDa, phosphorylated full-length TDP-43 of 45 kDa and high molecular smear, and a physiologic TDP-43 isoform at 43 kDa. No truncated isoform of TDP-43 at approximately 25 kDa or bands comparable to phosphorylated full-length TDP-43 were detected in CSF, suggesting that the pathologic signature of TDP-43 described in tissue fractions of FTLD-U and ALS seems not to be reflected in CSF.

Surprisingly, both monoclonal TDP-43 antibodies applied herein failed to detect protein bands in immunoblots of up to 200 μL of CSF. This could mean that the monoclonal antibodies did not bind to CSF TDP-43, possibly because of low affinity or epitope masking or that binding may be dependent on sample preparation procedure. To characterize the 45-kDa protein recognized by polyclonal antibodies in more detail, we applied N-terminus—and C-terminus—specific polyclonal antibodies. Whereas N-terminus—binding antibodies did not specifically recognize protein bands in 50 μL of CSF, C-terminus—specific antiserum detected the 45-kDa protein band in a similar fashion to the band seen with polyclonal antibodies raised against amino acids 1 through 260. The fact that the TDP-43 band in CSF migrated lower than

the full-length TDP-43 in mouse cells and brains, combined with the lack of staining using N-terminus—specific antibodies, suggests that the detected TDP-43 species in CSF is truncated at the N-terminus.

A yet undescribed protein band (to our knowledge) migrating at or above 20 kDa that reacts with C-terminus antibodies was present in CSF. Analysis of representative individuals from our diagnostic groups revealed that the 20-kDa TDP-43 fragment is generally present in CSF. The fact that TDP-43 can be found in CSF from individuals without dementia or ALS points to a physiologic process of TDP-43 release into the CSF.

TDP-43 levels in CSF tend to be elevated in disease states, which is in accord with increased TDP-43 gene expression in FTLD plus MND and in FTLD-U.[20] Relative quantification of 45-kDa bands was significantly different among our diagnostic groups (P=.046), based primarily on increased levels in diseased vs nondiseased individuals (P=.045). The absence of a correlation between TDP-43 levels in CSF and the CSF to serum albumin ratio points to an intrathecal origin of CSF TDP-43. On a statistical basis, determination of relative levels of 45-kDa TDP-43 allows discrimination of ALS and FTLD samples from controls (P=.03 and P=.02, respectively). The wide range of TDP-43 levels in FTLD CSF might be due to the heterogeneity of this disease group, which statistically consists of approximately 50% of patients without pathologic TDP-43 and 30% to 50% of patients with pathologic TDP-43. TDP-43 levels in ALS plus FTLD samples and in ALS plus DI samples were in the range of those of controls.

So far (to our knowledge), no disease specific markers for ALS or FTLD have been identified except genetic mutations pathogenic for ALS or FLTD.[11] Indeed, mutations in the TDP-43 gene (*TARDBP*) have been identified recently that are pathogenic for sporadic and familial ALS,[21,22] adding further credence to the view that mechanisms of neurodegeneration in ALS are linked to pathologic TDP-43.[3,11] Some studies[23-29] describe a slightly different total tau and phospho-tau protein profile, and varying levels of amyloid β (Aβ) 42, Aβ40, or Aβ38 are suggested to be characteristic of FTLD. In an analysis of FTLD subgroups, soluble tau was found to be reduced in dementia lacking distinctive histologic findings and in FTLD plus MND or MND—type inclusions.[30] In ALS, decreasing serum levels of S-100B protein were found in a follow-up investigation of ALS,[31,32] as well as elevated levels of neurofilament in the CSF.[33] However, these variations have limited differential diagnostic potential.

Our results suggest that disease-associated TDP-43 immunoreactivity in brain tissue is reflected by elevated levels of TDP-43 in CSF. Although we demonstrated a significant difference among the FTLD, ALS, and control groups, the TDP-43 immunoblot methods used herein did not distinguish among disease groups with differential diagnostic accuracy. Further CSF studies of additional patients (including those with postmortem-verified diagnoses) using quantitative TDP-43 assays (eg, enzyme-linked immunosorbent assay) are needed to determine if measurement of TDP-43 levels in CSF may be informative in multifactorial analyses with other biomarkers to aid in the diagnosis of different forms of FTLD and ALS during life, as this will expedite efforts to introduce specific disease-modifying therapies when they become available.

Acknowledgments

Funding/Support: This study was supported in part by grant P-LS-Prot/42 from the Landesstiftung Baden-Württemberg (Drs Steinacker and Otto), by grants cNeupro and NeuroTAS from the European Commission (Dr Otto), by grant 01GI0704 from the Federal Ministry of Education and Research (Drs Kretzschmar and Neumann) and the Heidelberger Akademie der Wissenschaften (Dr von Arnim), and by grants AG-10124 and AG-17586 from the National Institutes of Health (Drs Lee and Trojanowski).

Additional Contributions: We thank all the physicians who referred patients to our clinic.

REFERENCES

1. Kumar-Singh S, Van Broeckhoven C. Frontotemporal lobar degeneration: current concepts in the light of recent advances. Brain Pathol 2007;17(1):104–114. [PubMed: 17493044]
2. Josephs KA, Holton JL, Rossor MN, et al. Frontotemporal lobar degeneration and ubiquitin immunohistochemistry. Neuropathol Appl Neurobiol 2004;30(4):369–373. [PubMed: 15305982]
3. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314(5796):130–133. [PubMed: 17023659]
4. Arai T, Hasegawa M, Akiyama H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006;351(3):602–611. [PubMed: 17084815]
5. Johnson JK, Diehl J, Mendez MF, et al. Frontotemporal lobar degeneration: demographic characteristics of 353 patients. Arch Neurol 2005;62(6):925–930. [PubMed: 15956163]
6. Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. Neurology 2002;59(7):1077–1079. [PubMed: 12370467]
7. Ludolph AC, Sperfeld AD. Preclinical trials: an update on translational research in ALS. Neurodegener Dis 2005;2(34):215–219. [PubMed: 16909028]
8. Leigh PN, Swash M, Iwasaki Y, et al. Amyotrophic lateral sclerosis: a consensus viewpoint on designing and implementing a clinical trial. Amyotroph Lateral Scler Other Motor Neuron Disord 2004;5(2):84–98. [PubMed: 15204010]
9. Ringholz GM, Appel SH, Bradshaw M, Cooke NA, Mosnik DM, Schulz PE. Prevalence and patterns of cognitive impairment in sporadic ALS. Neurology 2005;65(4):586–590. [PubMed: 16116120]
10. Dickson DW, Josephs KA, Amador-Ortiz C. TDP-43 in differential diagnosis of motor neuron disorders. Acta Neuropathol 2007;114(1):71–79. [PubMed: 17569066]
11. Mackenzie IR, Bigio EH, Ince PG, et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with *SOD1* mutations. Ann Neurol 2007;61(5): 427–434. [PubMed: 17469116]
12. Kwong LK, Neumann M, Sampathu DM, Lee VM, Trojanowski JQ. TDP-43 proteinopathy: the neuropathology underlying major forms of sporadic and familial frontotemporal lobar degeneration and motor neuron disease. Acta Neuropathol 2007;114(1):63–70. [PubMed: 17492294]
13. Folstein MF, Folstein SE, McHugh PR. “Mini-Mental State”: a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189–198. [PubMed: 1202204]
14. Grön G, Bittner D, Schmitz B, Wunderlich AP, Riepe MW. Subjective memory complaints: objective neural markers in patients with Alzheimer’s disease and major depressive disorder. Ann Neurol 2002;51(4):491–498. [PubMed: 11921055]
15. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51(6):1546–1554. [PubMed: 9855500]
16. Pradat PF, Bruneteau G. Clinical characteristics of amyotrophic lateral sclerosis subsets [in French]. Rev Neurol (Paris 2006;162(spec No. 2):4S17–4S24. [PubMed: 17128085]
17. Zhang HX, Tanji K, Mori F, Wakabayashi K. Epitope mapping of 2E2-D3, a monoclonal antibody directed against human TDP 43. Neurosci Lett 2008;434(2):170–174. [PubMed: 18304732]
18. Snowden J, Neary D, Mann D. Frontotemporal lobar degeneration: clinical and pathological relationships. Acta Neuropathol 2007;114(1):31–38. [PubMed: 17569065]
19. Brandmeir NJ, Geser F, Kwong LK, et al. Severe subcortical TDP-43 pathology in sporadic frontotemporal lobar degeneration with motor neuron disease. Acta Neuropathol 2008;115(1):123–131. [PubMed: 18004574]
20. Mishra M, Paunesku T, Woloschak GE, et al. Gene expression analysis of frontotemporal lobar degeneration of the motor neuron disease type with ubiquitinated inclusions. Acta Neuropathol 2007;114(1):81–94. [PubMed: 17569064]
21. Gitcho MA, Baloh RH, Chakraverty S, et al. TDP-43 A315T mutation in familial motor neuron disease. Ann Neurol 2008;63(4):535–538. [PubMed: 18288693]
22. Sreedharan J, Blair IP, Tripathi VB, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008;319(5870):1668–1672. [PubMed: 18309045]

23. Bibl M, Mollenhauer B, Lewczuk P, et al. Validation of amyloid-β peptides in CSF diagnosis of neurodegenerative dementias. Mol Psychiatry 2007;12(7):671–680. [PubMed: 17339876]
24. Bibl M, Mollenhauer B, Wolf S, et al. Reduced CSF carboxyterminally truncated Aβ peptides in frontotemporal lobe degenerations. J Neural Transm 2007;114(5):621–628. [PubMed: 17245538]
25. Grossman M, Farmer J, Leight S, et al. Cerebrospinal fluid profile in frontotemporal dementia and Alzheimer's disease. Ann Neurol 2005;57(5):721–729. [PubMed: 15852395]
26. Hampel H, Teipel SJ. Total and phosphorylated tau proteins: evaluation as core biomarker candidates in frontotemporal dementia. Dement Geriatr Cogn Disord 2004;17(4):350–354. [PubMed: 15178952]
27. Riemenschneider M, Wagenpfeil S, Diehl J, et al. Tau and Aβ42 protein in CSF of patients with frontotemporal degeneration. Neurology 2002;58(11):1622–1628. [PubMed: 12058089]
28. Riemenschneider M, Wagenpfeil S, Vanderstichele H, et al. Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. Mol Psychiatry 2003;8(3):343–347. [PubMed: 12660807]
29. Rosso SM, Kaat L Donker, Baks T, et al. Frontotemporal dementia in the Netherlands: patient characteristics and prevalence estimates from a population-based study. Brain 2003;126(pt 9):2016–2022. [PubMed: 12876142]
30. Mott RT, Dickson DW, Trojanowski JQ, et al. Neuropathologic, biochemical, and molecular characterization of the frontotemporal dementias. J Neuropathol Exp Neurol 2005;64(5):420–428. [PubMed: 15892300]
31. Otto M, Bahn E, Wiltfang J, Boekhoff I, Beuche W. Decrease of S100 beta protein in serum of patients with amyotrophic lateral sclerosis. Neurosci Lett 1998;240(3):171–173. [PubMed: 9502231]
32. Süssmuth SD, Tumani H, Ecker D, Ludolph AC. Amyotrophic lateral sclerosis: disease stage related changes of tau protein and S100 beta in cerebrospinal fluid and creatine kinase in serum. Neurosci Lett 2003;353(1):57–60. [PubMed: 14642437]
33. Brettschneider J, Petzold A, Süssmuth S, Ludolph AC, Tumani H. Axonal damage markers in cerebrospinal fluid are increased in ALS. Neurology 2006;66(6):852–856. [PubMed: 16567701]

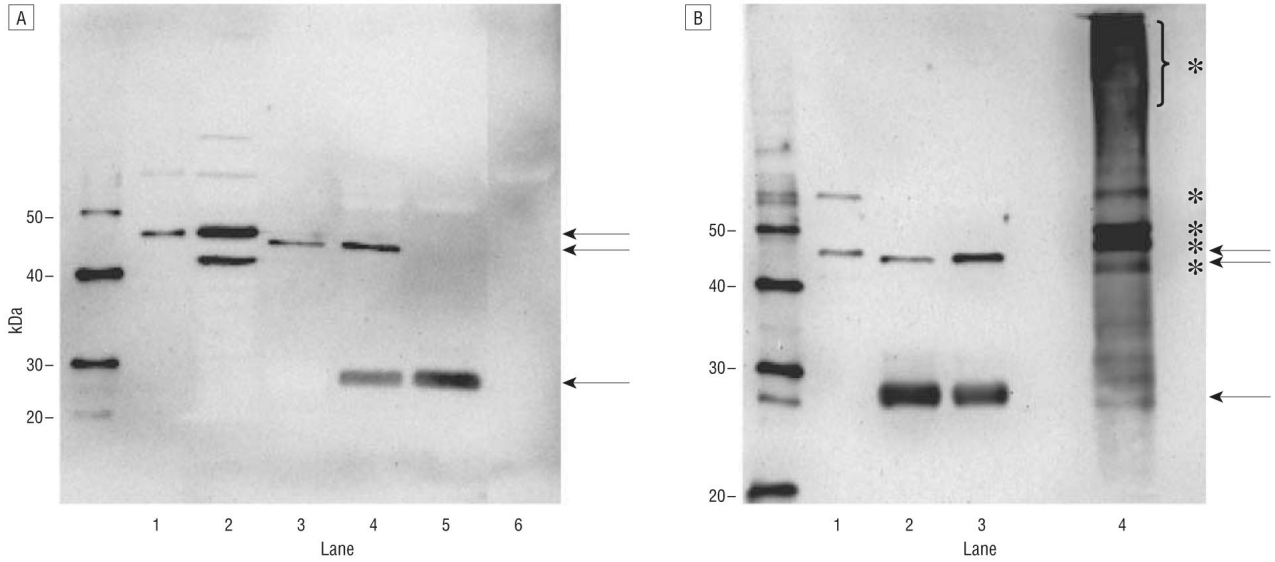

Figure 1.

Western immunoblot analyses of TAR DNA-binding protein 43 (TDP-43) applying rabbit polyclonal antibody. Lane 1 is the mouse RIPA (radioimmunoprecipitation assay) buffer homogenate. A band of approximately 46 kDa (upper arrow in A and B) was used as an internal standard for quantification of cerebrospinal fluid (CSF) 45-kDa TDP-43 bands (middle arrow in A and B). A, In mouse brain homogenate, a major band at 46 kDa and a minor band at 42 kDa are visible (lane 2). Lane 4 shows a representative signal in human CSF with a specific TDP-43 band at 45 kDa. A band at 28 kDa was found regularly in CSF (lower asterisk in A and B) but represents unspecific binding of IgG as demonstrated by depletion of CSF (lanes 3 and 4) and by purified human IgG (5 μg; lane 5, lower arrow). Purified human albumin (10 μg, lane 6) demonstrated no immunoreactive band. B, Immunodetection of TDP-43 and IgG in CSF (lanes 2 and 3, middle and lower arrows). In the urea fraction of brain tissue of frontotemporal lobar degeneration with ubiquitin-positive tau-negative inclusions using polyclonal TDP-43 antibody (lane 4), 2 major bands between 47 and 50 kDa and 2 minor bands at 44 and approximately 60 kDa are detected (4 lower asterisks). In addition, a high molecular mass smear is detected (lane 4, upper asterisk).

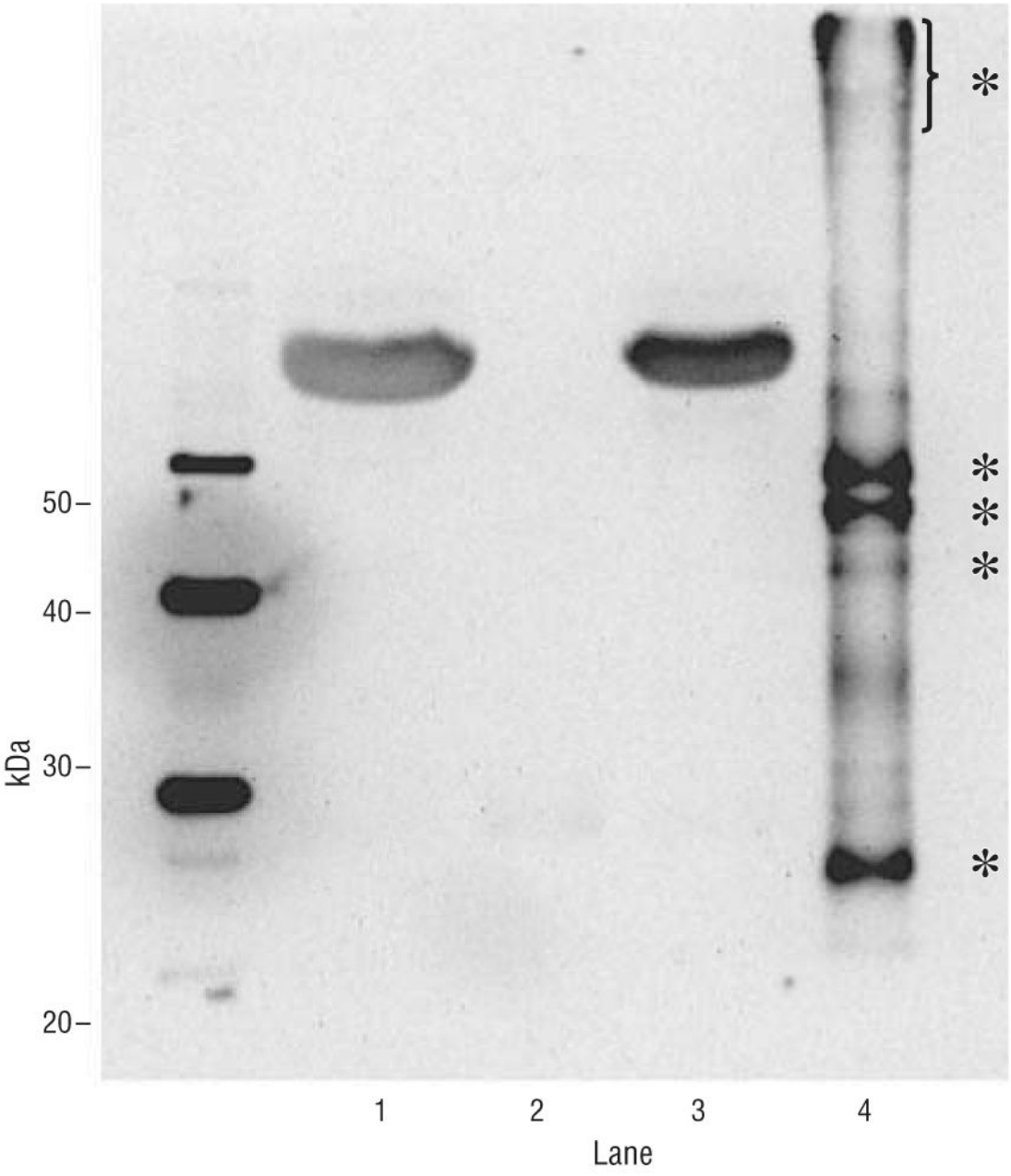

Figure 2.
TAR DNA-binding protein 43 (TDP-43) immunoblot applying monoclonal antibody (Proteintech Group Inc, Chicago, Illinois). In 2 cerebrospinal fluid samples (lanes 1 and 3) and in IgG (lane 2), no specific bands are detected. In the urea fraction of brain tissue of frontotemporal lobar degeneration with ubiquitin-positive tau-negative inclusions (FTLD-U), using monoclonal TDP-43 antibody, signature consisting of pathologically phosphorylated 46- to 50-kDa full-length TDP-43 (second and third asterisks), physiological 43-kDa TDP-43 (fourth asterisk), C-terminus-truncated 26-kDa TDP-43 (fifth asterisk), and a high relative molecular mass smear (first asterisk) are visible. In addition, a weak band at approximately 60 kDa can be seen.

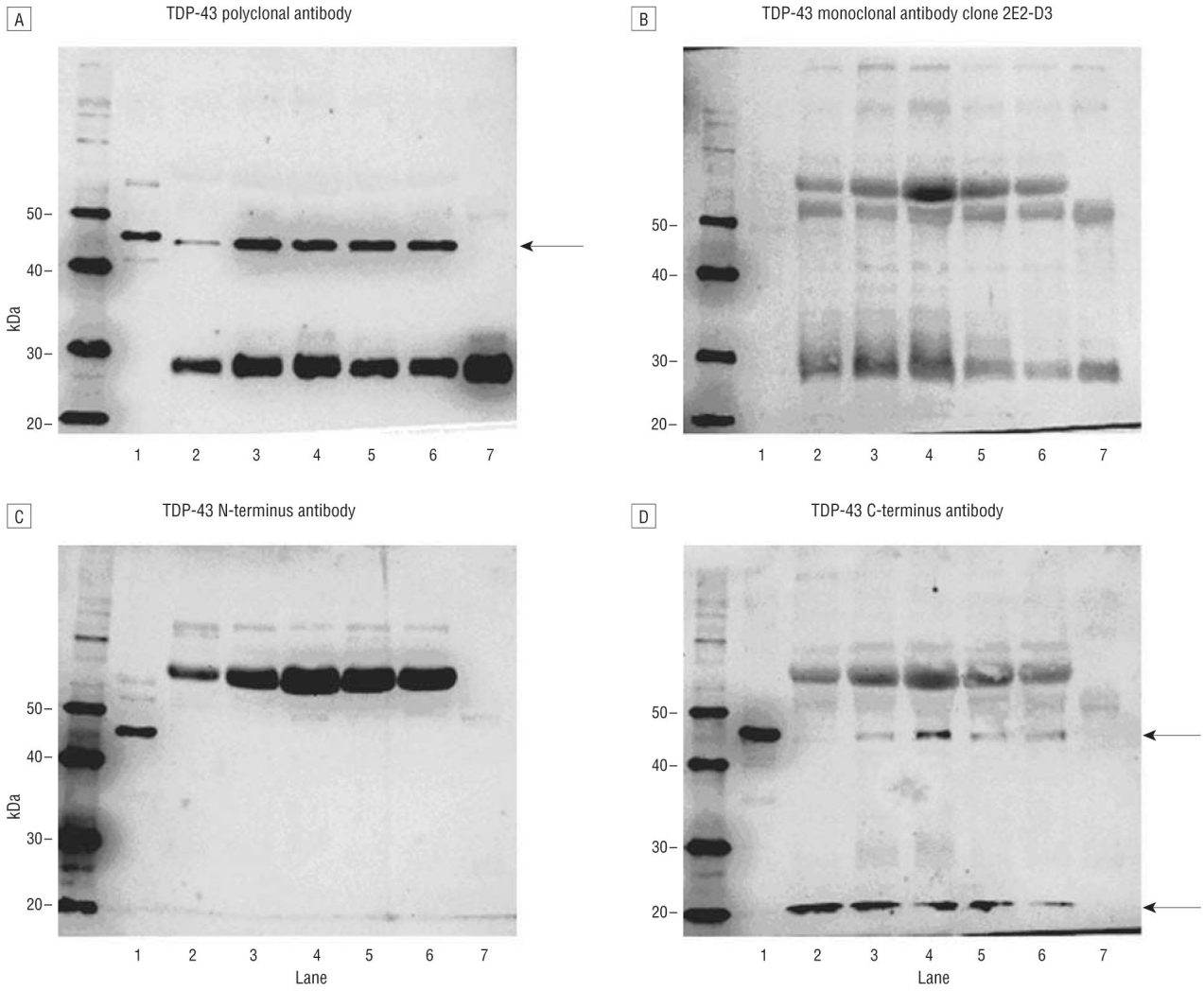

Figure 3.

Immunoblots of murine neuroblastoma cell homogenates (lane 1), IgG (lane 7), and 5 cerebrospinal fluid samples per blot (lanes 2-6) from the same individuals in the control group (lane 3), frontotemporal lobar degeneration (FTLD) group (lane 4), amyotrophic lateral sclerosis (ALS) plus FTLD group (lane 5), ALS plus additional signs of frontal disinhibition group (lane 6), and ALS group (lane 7). Immunodetection was performed using polyclonal TAR DNA-binding protein 43 (TDP-43) antibody (A) and monoclonal TDP-43 antibody clone 2E2-D3 (B), N-terminus—specific polyclonal antiserum (C), and C-terminus—specific polyclonal antiserum (D). Using polyclonal TDP-43 antibody, 45-kDa bands are detected in all patients (A, arrow). Neither monoclonal nor N-terminus—specific antibodies detect any specific bands (B and C), whereas bands at 45 kDa (D, upper arrow) and at approximately 20 kDa (D, lower arrow) are detected using C-terminus—specific antibodies.

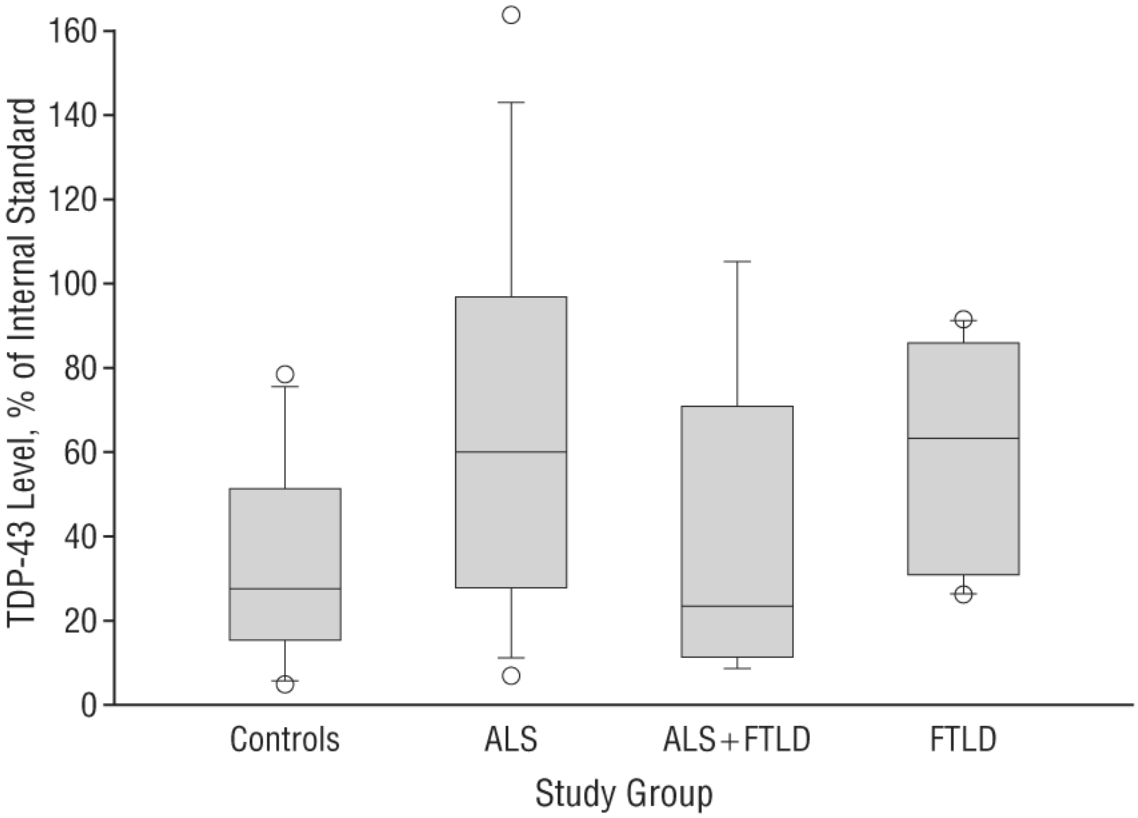

Figure 4.

Densitometric quantification of the 45-kDa TAR DNA-binding protein 43 (TDP-43) band recognized by polyclonal antibodies in immunoblots from 50 μL of cerebrospinal fluid samples from patients with amyotrophic lateral sclerosis (ALS) (n=15), ALS plus frontotemporal lobar degeneration (FTLD) (n=9), and FTLD (n=12) and from control subjects (n=13). The TDP-43 level is expressed in terms of percentages of an internal standard murine neuroblastoma cell preparation. Box plots show median values, 25% and 75% percentile values, 5% and 95% percentile values, and outliers.

Table 1

Number, Sex, and Age of Patients in the Study Groups at the Time of Lumbar Puncture

Study Group	No. of Patients	Female to Male Ratio	Age, Mean (SD) [Range], y
Control	13	7:6	60 (8.0) [48-74]
ALS	15	6:9	55 (6.6) [39-66]
ALS plus DI	3	3:0	63 (14.0) [47-74]
ALS plus FTLD	9	4:5	63 (7.1) [49-72]
FTLD	12	5:7	68 (8.6) [52-85]

Abbreviations: ALS, amyotrophic lateral sclerosis; DI, additional signs of frontal disinhibition; FTLD, frontotemporal lobar degeneration.

Table 2

Background Normalized Relative Values for the Intensity of 45-kDA Bands Detected in Immunoblots of Cerebrospinal Fluid Samples by Polyclonal TDP-43 Antibody

	Relative Intensity of 45-kDa TDP-43 Band, %	
Study Group	**Median**	**Mean (SD)**
Control	28	33 (24)
ALS	60	67 (45)
ALS plus DI	17	44 (48)
ALS plus FTLD	24	43 (36)
FTLD	63	60 (26)

Abbreviations: ALS, amyotrophic lateral sclerosis; DI, additional signs of frontal disinhibition; FTLD, frontotemporal lobar degeneration; TDP-43, TAR DNA-binding protein 43.