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Original Research Article

Cerebrospinal Fluid TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis Patients with and without the *C9ORF72* Hexanucleotide Expansion

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Key Words

Frontotemporal lobar degeneration · Frontotemporal dementia · Amyotrophic lateral sclerosis · TDP-43 · Cerebrospinal fluid · *C9ORF72* · Biomarker · ELISA

Abstract

Background: TDP-43 is the main protein component of ubiquitinated inclusions in a subgroup of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) patients. The *C9ORF72* hexanucleotide expansion is one of the main mutations associated with TDP-43 pathology in FTLD and ALS. Our aim was to analyze cerebrospinal fluid (CSF) TDP-43 levels and Alzheimer's disease biomarkers in FTLD and ALS patients and to test whether the *C9ORF72* expansion carrier status affects these variables. **Methods:** The patient cohort consisted of 90 clinically well-characterized FTLD (n = 69) and ALS (n = 21) patients. There were 30 patients with the *C9ORF72* expansion and 60 patients without the expansion. CSF TDP-43, A β_{1-42} , t-tau, and phospho-tau levels were measured using commercial ELISA kits. **Results:** There was no difference in CSF TDP-43 levels between the *C9ORF72* expansion carriers and the noncarriers. CSF TDP-43 levels were higher in ALS patients than in FTLD patients, and this finding was independent of the *C9ORF72* expansion carrier status. Males had significantly higher TDP-43 levels than females ($p = 0.008$ in the total cohort). **Conclusion:** CSF

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TDP-43 does not seem to distinguish the *C9ORF72* expansion carriers from noncarriers. However, higher CSF TDP-43 levels were detected in ALS than in FTLD, which might be an indicator of a more rapid progression of TDP-43 pathology in ALS.

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Introduction

Frontotemporal lobar degeneration (FTLD) is a genetically and neuropathologically heterogeneous group of syndromes. There are three clinically recognized subtypes: behavioral-variant frontotemporal dementia (FTD) with predominant behavioral and executive problems, and two language variants, namely progressive nonfluent aphasia and semantic dementia [1]. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder where the loss of motor neurons causes progressive weakness, fasciculations, and muscle atrophy. FTLD and ALS are overlapping syndromes, and about 15% of the patients with FTLD also develop concomitant ALS (FTLD-ALS) [2]. ALS patients may also show signs of cognitive impairment, usually a type of behavioral-variant FTD [3].

The molecular pathologies of FTLD and ALS show many similar pathological features. FTLD can be divided into five subtypes of which the two main differential pathological characteristics are FTLD-TDP (TDP-43 and ubiquitin-positive, tau-negative inclusions) and FTLD-tau (tau-positive inclusions) [4]. ALS is mainly associated with TDP-43 pathology, while tau pathology is not typical in ALS. The TDP-43 protein is normally expressed in many tissues, including the brain, and it has been identified as the major component of ubiquitin-positive inclusions in the brain of FTLD patients (as shown in our own series of autopsy cases) and ALS patients [5]. It is localized in the nucleus or shifts between the nucleus and the cytoplasm [6–8]. TDP-43 proteinopathies are characterized by insoluble neuronal cytoplasmic or intranuclear inclusions and glial cytoplasmic inclusions, which aggregate in the cells [9]. Cell death results in the release of TDP-43 and, therefore, the TDP-43 levels in cerebrospinal fluid (CSF) may increase.

There have only been a few previous reports on TDP-43 levels in FTLD and ALS patients with a known genetic background. Since the *C9ORF72* expansion is one of the most common genetic causes of FTLD and ALS with a TDP-43 pathology [10, 11] and is especially common in Finland [12], our aim was to improve the panel of possible biomarkers in the differential diagnostic process of FTLD, and we hypothesized that the *C9ORF72* expansion may have an effect on CSF TDP-43 levels. Furthermore, we have previously demonstrated that changes in CSF Alzheimer's disease (AD) biomarker levels ($A\beta_{1-42}$, t-tau, and phospho-tau) can be observed in patients carrying the *C9ORF72* expansion [13]. In this study, we investigated the CSF TDP-43 as well as the CSF AD biomarker levels in clinically well-characterized FTLD and ALS patients, including both *C9ORF72* expansion carriers and noncarriers.

Materials and Methods

Subjects and Samples

The patient cohort consisted of 90 clinically well-characterized FTLD (n = 69; 29 males) and ALS (n = 21; 10 males) patients, of whom 30 were *C9ORF72* expansion carriers and 60 were noncarriers (table 1).

The *C9ORF72* expansion (>45 repeats) was detected using a repeat-primed PCR [11]. *APOE* genotyping was done using a PCR-based method with the forward PCR primer 5'-GCA

Table 1. Clinical characteristics of the patients

	FTLD patients	ALS patients	Total patients	p value
Subjects	69 (76.7)	21 (23.3)	90	
Age, years	65.8±9.7	61.9±8.0	64.9±9.4	0.961
Male/female	29/40 (42/58)	10/11 (47.6/52.4)	39/51 (43.3/56.7)	0.008
MMSE	22.1±5.2	23.2±3.9	22.2±5.1	0.326
Missing cases, n	7	15	22	
APOE allele frequency ε2/3/4	0.06/0.70/0.24	0.06/0.76/0.18	0.06/0.72/0.22	0.260
Missing cases, n	20	4	24	
CSF TDP-43, pg/ml	3.0±1.0	4.1±1.3	3.2±1.2	0.001
CSF Aβ ₁₋₄₂ , pg/ml	647.7±244.9	762.9±302.2	674.6±262.2	0.134
CSF t-tau, pg/ml	362.7±269.7	315.4±124.3	351.7±243.9	0.910
CSF phospho-tau, pg/ml	64.8±35.3	48.4±14.2	61.0±32.3	0.147

Values are presented as n (%) or means ± SD unless otherwise specified.

CGG CTG TCC AAG GAG CTG CAG GC-3' and the reverse PCR primer 5'-GGC GCT CGC GGA TGG CGC TGA G-3' [14]. The CSF samples were obtained by lumbar puncture during the diagnostic procedure and stored in polypropylene tubes at -70 °C until the analysis. The study was approved by the ethics committees of the Kuopio and Oulu University Hospitals and followed the principles of the Declaration of Helsinki. All patients agreed to participate in the study, and blood and CSF samples were obtained after receiving written informed consent from patients and/or their legal representatives.

Measurements

The CSF TDP-43 levels were measured using a commercial ELISA (Cusabio, PR China) according to the manufacturer's protocol. The kit was tested first by measuring CSF samples not intended for this study, and there was a notable drift in the values measured. The beginning of the plate gave significantly higher values than the end of the plate. Therefore, the analyses were done by using half of the plate in each run, and the drift was acceptable. All samples were measured in triplicates. The CSF Aβ₁₋₄₂, t-tau, and phospho-tau levels were measured using a commercial ELISA (Innogenetics, Ghent, Belgium) according to the manufacturer's protocol. Samples were measured in duplicates, and the results were analyzed blind to diagnosis.

Statistical Analyses

Statistical analyses were performed using SPSS 21. Statistical significance was set at $p < 0.05$. The test of normality was done using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Statistical analyses were performed using a t test, the Mann-Whitney test, and the χ^2 test. Correlations were analyzed using Pearson's correlation test. All results are given as means ± SD, unless otherwise stated.

Results

The mean CSF TDP-43 level was 3.2 ± 1.2 pg/ml in the total cohort (table 1). There was no statistically significant difference in the mean CSF TDP-43 levels between the C9ORF72 expansion carriers and noncarriers in different diagnostic groups (tables 2, 3; fig. 1a, b). Female patients had lower CSF TDP-43 levels than male patients in the total cohort (2.9 ± 0.9 vs. 3.6 ± 1.4 pg/ml in females vs. males, respectively; $p = 0.008$), in the FTLD subcohort (2.7 ± 0.8 vs. 3.3 ± 1.2 pg/ml in females vs. males, respectively; $p = 0.029$), and in the ALS

Table 2. Clinical characteristics of the FTLD patients

	FTLD patients, <i>C9ORF72</i> expansion positive	FTLD patients, <i>C9ORF72</i> expansion negative	Total patients	p value
Subjects	20 (29.0)	49 (71.0)	69	
Age, years	63.3±8.4	61.9±8.0	65.8±9.7	0.915
Male/female	7/13 (35/65)	22/27 (44.9/55.1)	29/40 (42/58)	0.029
MMSE	24.9±3.2	21.0±5.5	22.1±5.2	0.140
Missing cases, n	3	4	7	
<i>APOE</i> allele frequency ϵ 2/3/4	0.05/0.82/0.13	0.07/0.63/0.30	0.06/0.70/0.24	0.0124
Missing cases, n	1	19	20	
CSF TDP-43, pg/ml	2.9±0.7	3.0±1.1	3.0±1.0	0.786
CSF $A\beta_{1-42}$, pg/ml	649.8±243.2	646.8±248.1	647.7±244.9	0.716
CSF t-tau, pg/ml	262.0±105.5	403.9±304.5	362.7±269.7	0.871
CSF phospho-tau, pg/ml	47.6±14.1	71.8±38.9	64.8±35.3	0.548

Values are presented as n (%) or means ± SD unless otherwise specified.

Table 3. Clinical characteristics of the ALS patients

	ALS patients, <i>C9ORF72</i> expansion positive	ALS patients, <i>C9ORF72</i> expansion negative	Total patients	p value
Subjects	10 (47.6)	11 (52.4)	21	
Age, years	58.6±6.5	64.9±8.3	61.9±8.0	0.117
Male/female	4/6 (40/60)	6/5 (54.5/45.5)	10/11 (47.6/52.4)	0.069
MMSE	29.0	22.0±2.9	23.2±3.9	0.199
Missing cases, n	9	6	15	
<i>APOE</i> allele frequency ϵ 2/3/4	0.06/0.81/0.12	0.06/0.72/0.22	0.06/0.76/0.18	0.407
Missing cases, n	2	2	4	
CSF TDP-43, pg/ml	4.6±1.5	3.7±1.0	4.1±1.3	0.116
CSF $A\beta_{1-42}$, pg/ml	794.1±283.6	734.6±329.2	762.9±302.2	0.425
CSF t-tau, pg/ml	260.1±147.6	365.7±73.9	315.4±124.3	0.215
CSF phospho-tau, pg/ml	39.3±7.8	56.6±13.9	48.4±14.2	0.642

Values are presented as n (%) or means ± SD unless otherwise specified.

subcohort (3.6 ± 0.9 vs. 4.7 ± 1.6 pg/ml in females vs. males, respectively; $p = 0.069$). There was no correlation between the CSF TDP-43 levels and the Mini-Mental State Examination (MMSE), age, or *APOE* ϵ 4 carrier or noncarrier status in the total cohort or in different clinical phenotypes.

Interestingly, we found that the CSF TDP-43 levels were significantly higher in ALS patients than in FTLD patients in the total cohort ($p = 0.001$; tables 1–3). A similar significant difference between the two diagnostic groups in the mean CSF TDP-43 levels was also found in patients with the *C9ORF72* expansion ($p = 0.003$).

In the total cohort, there were abnormal CSF $A\beta_{1-42}$ levels in 20%, abnormal CSF t-tau levels in 22%, and abnormal CSF phospho-tau levels in 24% of patients. The portions of abnormal biomarker levels in the subgroups are shown in table 4. All 3 AD biomarkers were abnormal in only 3 *C9ORF72* expansion noncarrier FTLD patients (4%). Furthermore, 16 FTLD patients (23%) had 1 abnormal biomarker, and 12 patients (17%) had 2 abnormal biomarkers. In the ALS group, 5 patients (24%) had 1 abnormal biomarker, and 3 patients

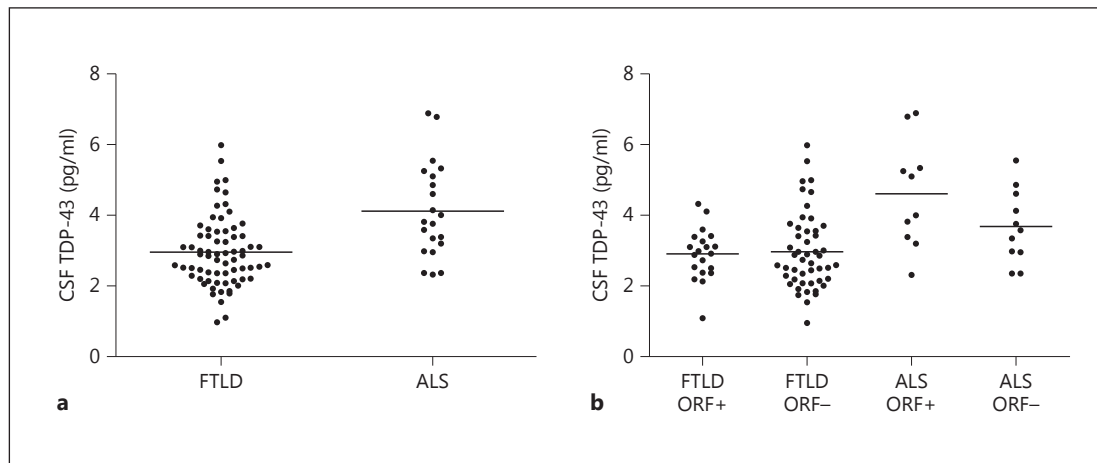


Fig. 1. **a** CSF TDP-43 levels in FTLD and ALS patients. **b** Different diagnostic groups and *C9ORF72* (ORF) expansion status. The mean levels are marked by lines.

Table 4. Abnormal CSF AD biomarker level portions in the total cohort and in the subcohorts

	Total cohort	FTLD patients (n = 69)	FTLD <i>C9ORF72</i> carriers (n = 20)	FTLD <i>C9ORF72</i> noncarriers (n = 49)	ALS patients (n = 21)	ALS <i>C9ORF72</i> carriers (n = 10)	ALS <i>C9ORF72</i> noncarriers
CSF A β_{1-42}	20%	22%	20%	22%	14%	30%	0%
CSF t-tau	22%	22%	5%	29%	24%	10%	36%
CSF phospho-tau	24%	28%	25%	29%	14%	0%	27%

(14%) had 2 abnormal biomarkers. There were no differences in the CSF A β_{1-42} , t-tau, and phospho-tau levels between the *C9ORF72* expansion carriers and noncarriers or the diagnostic subgroups. The CSF TDP-43 levels did not correlate with the CSF A β_{1-42} , t-tau, or phospho-tau levels in the total cohort nor in any subgroup either.

Discussion

Contrary to our hypothesis, we did not find any significant differences in the CSF TDP-43 levels between the *C9ORF72* expansion carriers and noncarriers. There is only one previous study concerning the CSF TDP-43 levels in genetically determined FTD patients (n = 25) [15]. However, there were only 2 *C9ORF72* expansion and 3 progranulin mutation carriers in that cohort; the 3 mutation carriers had higher CSF TDP-43 levels than the remaining FTD patients without known mutations. Our analysis in a larger sample (30 *C9ORF72* carriers vs. 60 noncarriers) does not support the previous finding. The contradictory findings may be due to a sample size that is still too small or the different methodology used to analyze the CSF TDP-43 levels.

We found that CSF TDP-43 was higher in patients with ALS than in patients with FTLD in the total cohort, and this phenotype-dependent correlation was also found in patients with the *C9ORF72* expansion. Similar findings concerning increased CSF TDP-43 levels in patients

with ALS compared to healthy controls have been found in a few previous studies [16–18]. One of these studies also included FTLD patients, and elevated CSF TDP-43 levels were also detected in this phenotype, while there was no difference between FTLD and ALS patients when using the immunoblot method [16]. The examined cohort was rather large ($n = 39$), but there was no information on the genetic background.

The reason for the higher CSF TDP-43 levels in ALS patients may be the faster neurodegeneration and disease progression in this patient group. The survival time after the diagnosis of ALS is usually 3–4 years, whereas FTLD patients may have a survival time of more than 10 years. The more rapid neurodegeneration in ALS may release intracellular TDP-43 from inclusions, which may lead to elevated TDP-43 levels in the CSF compared to FTLD. According to this hypothesis, CSF TDP-43 levels would be higher in patients with a more rapid progression of the disease. In the present study, survival data are not yet available, and it has to be noted that opposite results have also been presented. Noto et al. [18] found that, in patients with ALS, the highest levels of TDP-43 were detected in cases with a slower progression of the disease.

The origin of TDP-43 in the CSF has also been speculated on. In a small cohort ($n = 13$) of FTLD and ALS patients without known genetic background, Feneberg et al. [19] studied the levels of TDP-43 in the CSF, lymphocytes, and serum by one- and two-dimensional Western blotting and quantitative mass spectrometry. They found, surprisingly, that the TDP-43 in the CSF mainly originated from blood and, thus, CSF or blood TDP-43 levels may not be a useful diagnostic tool.

We found that CSF TDP-43 levels were higher in men in the total cohort and in both the FTLD and the ALS subcohorts. In previous studies, no differences in the TDP-43 levels between men and women have been detected. The incidence and prevalence of ALS is higher in men than in women. The onset of the disease is also earlier in men, but differences in survival time have not been detected [20]. The reason for these differences is unknown, but they may be due to differences between the male and female nervous system and differences in the ability to repair damage [20]. According to this notion, it is possible that the differences in the TDP-43 levels of men and women are caused by gender-specific differences in the nervous system and in the ability to process TDP-43.

In our previous study, we investigated CSF AD biomarker levels ($A\beta_{1-42}$, t-tau, and phospho-tau) in patients with the *C9ORF72* expansion, and we found decreased CSF $A\beta_{1-42}$ levels in 25% of the cases [13]. A similar profile of AD biomarkers in patients with the *C9ORF72* expansion was found in the present study in both clinical phenotypes (FTLD and ALS). Changes in t-tau and phospho-tau were also detected in a significant proportion of patients with and without the *C9ORF72* expansion. However, there was no difference in the CSF AD biomarker levels between *C9ORF72* expansion carriers and noncarriers in the different diagnostic groups. A relatively high number of FTLD patients without the *C9ORF72* expansion may represent mixed or tau-pathology, which may have been reflected in the elevation of CSF t-tau and phospho-tau levels [21, 22].

There was no correlation between the *APOE* genotype and the CSF TDP-43 level. In the present study, the frequency of *APOE* $\epsilon 4$ was higher in *C9ORF72* expansion noncarriers than in carriers. However, the frequency of *APOE* $\epsilon 4$ in the noncarriers is the same as in the general Finnish population [23]. Interestingly, the frequency of *APOE* $\epsilon 4$ in the FTLD patients with the *C9ORF72* expansion was remarkably low.

In conclusion, CSF TDP-43 levels seem to be associated with ALS and may be a marker of more rapid progression of diseases presenting with TDP-43 pathology. However, CSF TDP-43 does not seem to distinguish *C9ORF72* expansion carriers from noncarriers.

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