

# Cerebrospinal Fluid Tau, p-Tau 181 and Amyloid- $\beta_{38/40/42}$ in Frontotemporal Dementias and Primary Progressive Aphasias

Mirko Bibl<sup>a</sup> Brit Mollenhauer<sup>c</sup> Piotr Lewczuk<sup>d</sup> Hermann Esselmann<sup>b</sup>  
Stefanie Wolf<sup>e</sup> Markus Otto<sup>f</sup> Johannes Kornhuber<sup>d</sup> Eckart Rütter<sup>g</sup>  
Jens Wiltfang<sup>b</sup>

<sup>a</sup>Department of Psychiatry, Psychotherapy and Addiction Medicine, Kliniken Essen-Mitte, University of Duisburg-Essen, and <sup>b</sup>Klinik für Psychiatrie und Psychotherapie der Rheinischen Kliniken Essen, Universität Duisburg-Essen, Essen, <sup>c</sup>University of Göttingen and Paracelsus-Elena Klinik, Kassel, <sup>d</sup>Department of Psychiatry and Psychotherapy, University of Erlangen-Nuremberg, Erlangen, <sup>e</sup>Department of Psychiatry, University of Göttingen, Göttingen, <sup>f</sup>Institute for Neurology, University of Ulm, Ulm, and <sup>g</sup>Department of Psychiatry and Psychotherapy, Ludwig-Maximilian-University of Munich, Munich, Germany

## Key Words

Frontotemporal dementias · Primary progressive aphasias · Alzheimer's dementia · Cerebrospinal fluid · Amyloid- $\beta$  peptides · Biomarkers · Tau · p-Tau

## Abstract

**Background/Aims:** We determined cerebrospinal fluid (CSF) concentrations of amyloid- $\beta$  ( $A\beta_{1-38}$ ,  $A\beta_{1-40}$ ,  $A\beta_{1-42}$ , total tau and phospho-tau (p-tau) in order to study their differential expression in frontotemporal dementia (FTD,  $n = 25$ ) and primary progressive aphasia (PPA,  $n = 12$ ) as compared to Alzheimer's dementia (AD,  $n = 25$ ) and nondemented controls ( $n = 20$ ). **Methods:** Commercially available ELISA and electrochemiluminescence methods were applied. **Results:** High CSF p-tau and low ratios of  $A\beta_{1-42}/A\beta_{1-40}$  and  $A\beta_{1-42}/A\beta_{1-38}$ , respectively, were specific for AD. CSF  $A\beta_{1-38}$  was reduced in FTD as compared to each of the other diagnostic groups, including PPA. CSF tau and p-tau levels were elevated in PPA as compared to FTD. **Conclusion:** This is the first detailed report on biomarker patterns in PPA, indicating

distinct CSF biomarker patterns in FTD and PPA as major subgroups of frontotemporal lobar degeneration. The diagnostic and pathophysiological implications of our results warrant further studies on larger and neuropathologically diagnosed patient populations.

Copyright © 2010 S. Karger AG, Basel

## Introduction

Over the past 10 years, characteristic cerebrospinal fluid (CSF) biomarker patterns have been established to support the clinical diagnosis of Alzheimer's dementia (AD). The main protagonists are amyloid- $\beta$  ( $A\beta_{1-42}$ , tau and its phosphorylated forms (e.g. p-tau 181). In contrast, the literature about specific biomarkers for other common neurodegenerative disorders, such as frontotemporal lobar degeneration (FTLD), is sparse. FTLD is defined as a generic term to summarize heterogeneous clinical phenotypes that may appear sporadically or be caused by various genetic mutations. There is also great heterogene-

ity in neuropathologic findings, but the present study focuses on the clinical definition of diagnostic groups. Three sets of criteria for clinically diagnosing FTLD have been published so far. The clinical criteria by Neary et al. [1] are most helpful when focussing on the 3 prototypic clinical subtypes of FTLD: frontotemporal dementia (FTD), primary progressive aphasia (PPA) and semantic dementia. However, clinical diagnosis among these subgroups often remains crucial in a definite case. Thus, biomarkers for applicable diagnostic testing are warranted and still deserve intensive investigation. There are previous reports about the aforementioned AD-specific biomarkers in FTLD and FTD, revealing rather unspecific alterations [2–4]. Two reports have focussed on  $A\beta_{1-40}$  in FTLD, one of which showed a decrease in comparison to controls and AD [5]. The other did not include a comparison group, but reported an inverse correlation of CSF  $A\beta_{1-40}$  with frontal lobe volume in 11 FTD patients [6]. Our group reported decreased  $A\beta_{1-37}$  and  $A\beta_{1-38}$  levels as part of disease-specific CSF  $A\beta$  peptide patterns in FTLD and FTD [7, 8], using the  $A\beta$ -SDS-PAGE with Western immunoblot ( $A\beta$ -SDS-PAGE/immunoblot). Interestingly, electrochemiluminescence measurements confirmed decreased CSF  $A\beta_{1-38}$  levels in FTD, but with considerably lower test accuracy for diagnosing the disease in a single case [9]. Other biomarker findings for FTD include slightly elevated levels of TAR DNA-binding protein 43 and differences in processing soluble ectodomain of the amyloid precursor protein [10, 11].

PPA represents another major subgroup of FTLD, but very little is known about its CSF biomarker patterns. This prompted us to determine the major  $A\beta$  species in CSF 1–40, 1–38 and 1–42 in addition to total tau and p-tau 181 in patients with FTD ( $n = 25$ ) and PPA ( $n = 12$ ), as compared to AD ( $n = 25$ ) and nondemented disease controls (NDC,  $n = 20$ ).

## Patients and Methods

Eighty-two CSF samples were referred to our laboratory between 1999 and 2004 and investigated. CSF concentrations of  $A\beta_{1-38}$  ( $n = 82$ ),  $A\beta_{1-40}$  ( $n = 78$ ),  $A\beta_{1-42}$  ( $n = 73$ ), p-tau 181 ( $n = 78$ ) and tau ( $n = 58$ ) were measured. Aliquots of these samples had been studied previously under another objective, focussing on a distinct issue of differentially diagnosing dementias [8]. CSF of AD and other dementia and depressive patients came from the memory clinic of the University of Göttingen or from wards. Cognitive impairment was assessed at least by Mini-Mental State Examination (MMSE). More detailed neuropsychological testing including clock drawing and the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) test battery was additionally

carried out on the majority of patients with cognitive complaints (46/72). Specifically, 5/10, 10/25, 21/25 and 10/12 patients underwent neuropsychological testing in the groups of depressive cognitive complainers (DCC), and AD, FTD and PPA patients, respectively. Detailed neuropsychological assessment was hindered in 2 patients with FTD and 2 patients with PPA by severe lingual or cognitive deficits.

A psychiatrist, a neurologist and a neuropsychologist rendered diagnoses based on a thorough medical history, clinical examination, neuropsychological assessment, clinical records and best clinical judgment. The investigators were blinded to the neurochemical outcome measures. Investigations were carried out with the informed consent of patients or their authorized caregivers. The study was conducted under the guidelines of the Declaration of Helsinki [12] and approved by the ethics committee of the University of Göttingen.

### Patients

**Nondemented Disease Controls.** The NDC group consisted of 2 subgroups: (1) patients with peripheral neurological diseases (PND) without organic brain affection, and (2) DCC. The 10 patients in the PND subgroup underwent lumbar puncture to investigate central nervous affection in case of polyneuropathy ( $n = 7$ ), benign paroxysmal positioning vertigo ( $n = 1$ ), intervertebral disk herniation ( $n = 1$ ), autosomal dominant hereditary spastic spinal palsy ( $n = 1$ ). The 10 DCC underwent lumbar puncture for differential diagnosis of cognitive complaints during the course of disease. The diagnosis of depression was made according to the criteria of the DSM-IV, and cognitive impairment was assessed at least by the MMSE. Patients with persistent cognitive decline for more than 6 months, an MMSE score below 26 or clear focal atrophy in brain imaging (CT or MRI) were excluded.

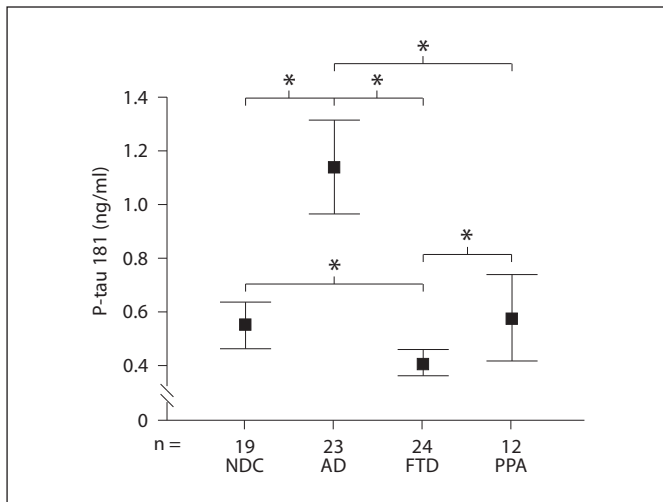
**Patients with AD.** Twenty-five patients fulfilled the DSM-IV criteria and NINCDS-ADRDA criteria for clinical diagnosis of AD [13]. Structural (CT or MRI) or functional (single-photon-emission CT or positron emission tomography) brain imaging displayed global cortical atrophy, or temporal, parietotemporal or frontotemporal focal atrophy, or marked hypometabolism of these regions.

**Patients with FTD and PPA.** FTD ( $n = 25$ ) and PPA ( $n = 12$ ) were diagnosed according to the consensus criteria [1]. Structural (CT or MRI) or functional (single-photon-emission CT or positron emission tomography) brain imaging revealed frontal or frontotemporal focal atrophy or marked hypometabolism in case of FTD, and left anterior temporal focal atrophy or marked hypometabolism in case of PPA.

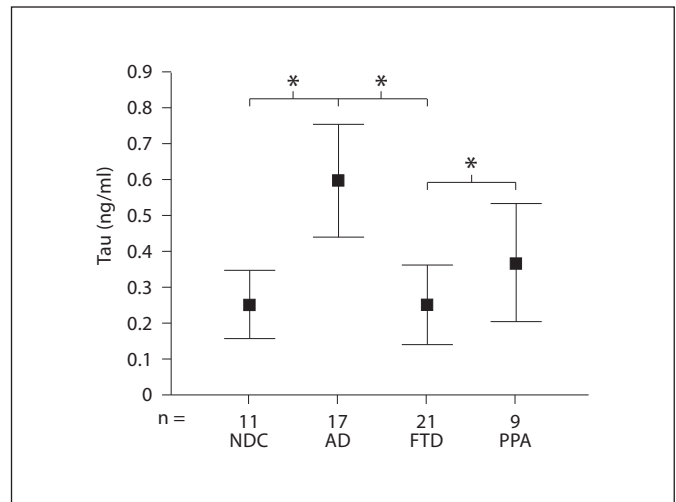
### Test Methods

**Preanalytical Treatment of CSF.** CSF was drawn by lumbar puncture into polypropylene vials and centrifuged (1,000 g, 10 min, 4°C). Aliquots of 200  $\mu$ l were kept at room temperature for a maximum of 24 h before storage at  $-80^{\circ}\text{C}$  for subsequent p-tau,  $A\beta_{1-38}$  and  $A\beta_{1-40}$  analysis. CSF for  $A\beta_{1-42}$  and total tau ELISA analysis was stored at  $+4^{\circ}\text{C}$  and analyzed within 2 days.

**ELISA for p-Tau, Total Tau Protein and  $A\beta_{1-42}$  and  $A\beta_{1-40}$ .** ELISA for p-tau at Thr181 was conducted as previously described [14]. Briefly, the HT7 monoclonal antibody (MAb) directed against both normal tau and p-tau was used for capturing, and biotinylated MAb AT270 for detection. According to published standard methods [15], the Innostest hTAU Antigen ELISA and



**Fig. 1.** Means and 95% CI of CSF p-tau concentrations for each diagnostic group. \*  $p < 0.05$ .



**Fig. 2.** Means and 95% CI of CSF tau concentrations for each diagnostic group. \*  $p < 0.05$ .

Innotest  $\beta$ -Amyloid<sub>1-42</sub> (ELISA Innogenetics, Ghent, Belgium) served for quantification of CSF tau and  $A\beta_{1-42}$ , respectively. For the quantification of  $A\beta_{1-40}$ , the ELISA of The Genetics Company was conducted similarly, except that before application to a microtiter plate, the samples were prediluted 1:5 with diluting buffer.

**Electrochemiluminescence Detection of  $A\beta_{1-38}$ .** Electrochemiluminescence detection of  $A\beta_{1-38}$  in CSF was performed according to the manufacturer's recommendations (Meso Scale Discovery). In brief, Multi-Spot 4-spot 96-well plates, precoated with MAb 6E10, were blocked with solution A. The plate was then incubated with a reference peptide dilution series or 100  $\mu$ l CSF for 1 h, followed by incubation with C-terminal Sulfo-Tag  $A\beta_{1-38}$  antibody and finally Read Buffer, for 1 h each. Washing steps with 1  $\times$  Tris buffer were performed in between. The emitted light was measured at approximately 620 nm.

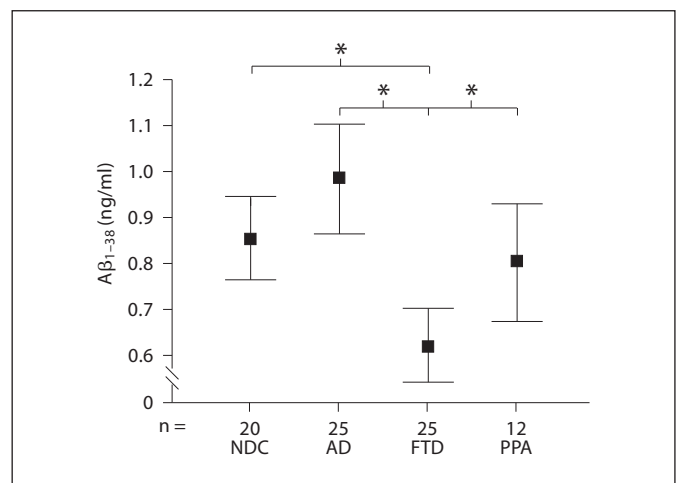
#### Statistical Analysis

$A\beta$  peptides, tau and p-tau were expressed as absolutes (in nanograms per milliliter). Patient groups were characterized by mean and SD. The Mann-Whitney U test was employed for comparisons of diagnostic groups. Correlations were estimated by Spearman's rho. The two-sided level of significance was taken as  $p < 0.05$ . The statistical software package SPSS, version 12.0, served for computations.

## Results

### Test Results

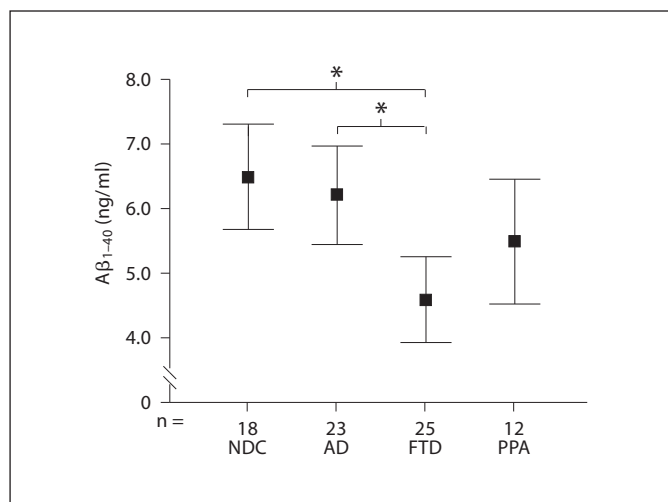
The mean age of FTD and PPA patients was significantly below that of the AD group ( $p < 5 \times 10^{-2}$ ). Otherwise, there was no significant difference in age among the diagnostic groups. The mean MMSE score did not sig-



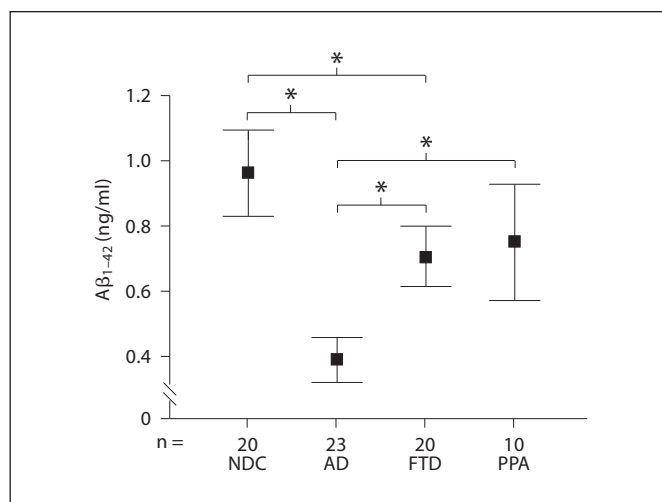
**Fig. 3.** Means and 95% CI of CSF  $A\beta_{1-38}$  concentrations for each diagnostic group. \*  $p < 0.05$ .

nificantly differ between the dementia groups. There was no significant difference among PND patients and DCC in any of the parameters investigated. For the following statistical evaluation, the 2 groups were therefore considered as one (NDC). The respective CSF concentrations underlying the following comparisons are displayed in figures 1-5.

**Neurochemical Phenotype of AD versus NDC.**  $A\beta_{1-42}$  was selectively decreased in AD ( $p = 1.3 \times 10^{-7}$ ), where-



**Fig. 4.** Means and 95% CI of CSF A $\beta_{1-40}$  concentrations for each diagnostic group. \*  $p < 0.05$ .



**Fig. 5.** Means and 95% CI of CSF A $\beta_{1-42}$  concentrations for each diagnostic group. \*  $p < 0.05$ .

**Table 1.** Total and p-tau, and A $\beta$  peptides 1–38, 1–40 and 1–42 in CSF of diagnostic groups

	NDC (n = 20)	AD (n = 25)	FTD (n = 25)	PPA (n = 12)
Age, years	65.05 $\pm$ 9.4	71.56 $\pm$ 8.6	58.92 $\pm$ 10.2	62.33 $\pm$ 10.1
Sex (m/w), n	11/9	5/20	17/8	10/2
In-/outpatients, n	10/10	10/15	13/12	6/6
MMSE score	28.27 $\pm$ 1.7	17.06 $\pm$ 6.2	19.74 $\pm$ 10.1	17.50 $\pm$ 12.8
p-tau ELISA 1, ng/ml	0.055 $\pm$ 0.02	0.114 $\pm$ 0.04	0.041 $\pm$ 0.01	0.058 $\pm$ 0.03
Tau ELISA 1, ng/ml	0.252 $\pm$ 0.14	0.601 $\pm$ 0.31	0.252 $\pm$ 0.25	0.370 $\pm$ 0.22
A $\beta_{1-38}$ <sup>1</sup> , ng/ml	0.857 $\pm$ 0.20	0.984 $\pm$ 0.28	0.623 $\pm$ 0.20	0.806 $\pm$ 0.20
A $\beta_{1-40}$ <sup>2</sup> , ng/ml	6.492 $\pm$ 1.63	6.225 $\pm$ 1.82	4.603 $\pm$ 1.60	5.503 $\pm$ 1.51
A $\beta_{1-42}$ <sup>2</sup> , ng/ml	0.971 $\pm$ 0.30	0.384 $\pm$ 0.16	0.709 $\pm$ 0.20	0.754 $\pm$ 0.26
A $\beta_{1-38}$ /A $\beta_{1-40}$ , ng/ml	0.134 $\pm$ 0.02	0.165 $\pm$ 0.03	0.143 $\pm$ 0.04	0.155 $\pm$ 0.06
A $\beta_{1-42}$ /A $\beta_{1-38}$ , ng/ml	1.177 $\pm$ 0.39	0.377 $\pm$ 0.16	1.198 $\pm$ 0.43	1.009 $\pm$ 0.39
A $\beta_{1-42}$ /A $\beta_{1-40}$ , ng/ml	0.158 $\pm$ 0.05	0.064 $\pm$ 0.02	0.158 $\pm$ 0.07	0.148 $\pm$ 0.06

<sup>1</sup> A $\beta$  peptide concentrations as measured by electrochemiluminescence detection (Meso Scale Discovery).

<sup>2</sup> A $\beta$  peptide concentrations as measured by ELISA.

as A $\beta_{1-40}$  levels were virtually unchanged between the 2 groups. A $\beta_{1-38}$  tended to be increased in AD, but missed the level of significance. Consequently, the ratios of A $\beta_{1-42}$ /A $\beta_{1-40}$  ( $p = 8.0 \times 10^{-10}$ ) and A $\beta_{1-42}$ /A $\beta_{1-38}$  ( $p = 2.0 \times 10^{-8}$ ) showed a decrease in AD. A $\beta_{1-38}$ /A $\beta_{1-40}$  was significantly elevated in AD (table 1). CSF total tau ( $p = 2.4 \times 10^{-4}$ ) and p-tau ( $p = 1.4 \times 10^{-6}$ ) were elevated in AD.

*Neurochemical Phenotype of FTD versus NDC.* FTD patients showed lower levels of A $\beta_{1-38}$  ( $p = 4.1 \times 10^{-4}$ ), A $\beta_{1-42}$  ( $p = 3.2 \times 10^{-3}$ ) and A $\beta_{1-40}$  ( $p = 9.7 \times 10^{-4}$ ) than NDC. The A $\beta$  ratios remained virtually unchanged between FTD and NDC. CSF total tau did not significantly differ, whereas p-tau was lowered in FTD ( $p = 5.3 \times 10^{-3}$ ).

*Neurochemical Phenotype of FTD versus AD.* In comparison to AD, FTD showed lower levels of A $\beta_{1-38}$  ( $p = 1.9 \times 10^{-5}$ ) and A $\beta_{1-40}$  ( $p = 4.5 \times 10^{-3}$ ), but higher lev-

els of  $A\beta_{1-42}$  ( $p = 8.7 \times 10^{-7}$ ). Consequently, the ratios of  $A\beta_{1-42}/A\beta_{1-40}$  ( $p = 2.8 \times 10^{-7}$ ) and  $A\beta_{1-42}/A\beta_{1-38}$  ( $p = 1.9 \times 10^{-8}$ ) were decreased in AD, while  $A\beta_{1-38}/A\beta_{1-40}$  was lowered in FTD ( $p = 2.3 \times 10^{-2}$ ) (table 1). CSF total tau ( $p = 6.3 \times 10^{-6}$ ) and p-tau ( $p = 4.2 \times 10^{-9}$ ) were elevated in AD.

*Neurochemical Phenotype of PPA versus NDC.* PPA displayed lower overall  $A\beta$  levels ( $p = 4.5 \times 10^{-2}$ ). There was a tendency toward lower levels of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  than in NDC, which missed the level of significance. The  $A\beta$  ratios as well as tau and p-tau levels were unaltered.

*Neurochemical Phenotype of PPA versus AD.* In comparison to AD, PPA displayed a tendency toward lower levels of  $A\beta_{1-38}$ , but this missed the level of significance.  $A\beta_{1-40}$  did not differ significantly, but  $A\beta_{1-42}$  was decreased in AD ( $p = 2.0 \times 10^{-4}$ ). Consequently, the ratios of  $A\beta_{1-42}/A\beta_{1-40}$  ( $p = 5.9 \times 10^{-5}$ ) and  $A\beta_{1-42}/A\beta_{1-38}$  ( $p = 1.0 \times 10^{-6}$ ) were decreased in AD. In contrast, the ratio of  $A\beta_{1-38}/A\beta_{1-40}$  was lowered in PPA ( $p = 4.5 \times 10^{-2}$ ). CSF p-tau ( $p = 6.2 \times 10^{-6}$ ), but not total tau, was significantly elevated in AD.

*Neurochemical Phenotype of FTD versus PPA.* FTD patients showed lower levels of  $A\beta_{1-38}$  ( $p = 1.6 \times 10^{-2}$ ) than PPA patients, whereas  $A\beta_{1-42}$  and  $A\beta_{1-40}$  were unaltered between these 2 diagnostic groups. The  $A\beta$  ratios remained virtually unchanged too. CSF total tau ( $p = 4.0 \times 10^{-2}$ ) and p-tau ( $p = 1.8 \times 10^{-2}$ ) were elevated in PPA.

### Correlations

Throughout all investigated patient groups, the different  $A\beta$  peptides were strongly correlated with each other, except for NDC and FTD, where a correlation of  $A\beta_{1-42}$  to  $A\beta_{1-40}$  and  $A\beta_{1-38}$ , respectively, was lacking. Tau and p-tau were negatively correlated with  $A\beta_{1-42}$ . We found higher tau levels in younger AD patients, but levels of p-tau and tau increased with age in NDC. Otherwise, none of the markers investigated, alone or in combination, were correlated with sex, age or severity of dementia in any of the groups investigated. All reported correlations reached the level of significance ( $p < 0.05$ ).

### Discussion

In order to investigate disease-specific biomarker patterns in FTLD subgroups, we evaluated CSF  $A\beta$  peptides 1–40, 1–38 and 1–42 as well as total tau and p-tau 181 in patients with FTD and PPA in comparison with AD patients and NDC.

The NDC group consisted of two subgroups, one with PND, the other DCC, which did not differ in any of the parameters investigated. The results of the AD group as compared to the NDC confirm our own previous results [8, 16] and those from studies of others [17]. The overlapping CSF tau and  $A\beta_{1-42}$  levels between AD and the other dementia groups indicate a limited differential diagnostic value of these biomarkers.

### *A $\beta$ Peptide Patterns in FTD*

FTD patients presented with low levels of  $A\beta_{1-38}$  as compared to NDC. Using electrochemiluminescence as an independent method, these results confirm our findings from the  $A\beta$ -SDS-PAGE/immunoblot [7, 8]. Although less pronounced, CSF  $A\beta_{1-40}$  was reduced in FTD as measured by electrochemiluminescence. In previous studies, the  $A\beta$ -SDS-PAGE/immunoblot revealed no significant change in  $A\beta_{1-40}$ , which may be due to methodological differences [9]. Conflicting results of  $A\beta_{1-40}$  in FTD also arise from other studies. A recent study found a significant decrease in  $A\beta_{1-40}$  in FTLD compared with AD and control subjects [5]. Another study reported a linear correlation between CSF  $A\beta_{1-40}$  levels and the degree of frontal atrophy as measured by MRI in FTD, suggesting higher CSF  $A\beta_{1-40}$  levels in FTD patients with more pronounced frontal atrophy [6]. However, the results of the latter study are limited by the lack of a control group, which hampers a direct comparison to our results and those of others. The moderate decrease in  $A\beta_{1-42}$  in FTD as compared to NDC in the present study confirms our previous findings by  $A\beta$ -SDS-PAGE/immunoblot [7, 8], as well as results from ELISA-based investigations of others [3, 17].

In comparison to AD, FTD displayed lower levels of  $A\beta_{1-38}$  and  $A\beta_{1-40}$  accompanied by a less pronounced decrease in  $A\beta_{1-42}$ . Accordingly, FTD could be differentiated from AD by either a decreased ratio of  $A\beta_{1-38}/A\beta_{1-40}$  or increased ratios of  $A\beta_{1-42}/A\beta_{1-40}$  and  $A\beta_{1-42}/A\beta_{1-38}$ , respectively. These results match those of our previous reports [7–9] and the study by Pijnenburg et al. [5]. They underline the diagnostic usefulness not only of the ratios of  $A\beta_{1-42}/A\beta_{1-40}$  and  $A\beta_{1-42}/A\beta_{1-38}$ , but also of the ratio of  $A\beta_{1-38}/A\beta_{1-40}$  in differentiating FTD from AD. Within these ratios, decreased CSF  $A\beta_{1-38}$  may be considered as the most specific parameter of FTD as moderately diminished concentrations of  $A\beta_{1-42}$  and  $A\beta_{1-40}$  could also be found in other dementias such as dementia with Lewy bodies [18] and vascular dementia [19].

### *Tau and p-Tau Proteins in FTD*

The results of unchanged CSF tau levels and mildly decreased p-tau levels in FTD as compared to controls are in line with previous studies by others [17, 20]. The decrease in CSF p-tau in FTD as compared to controls may be explained by disturbances of tau phosphorylation regulatory mechanisms in FTD [20]. Another explanation may be an underlying AD pathology in NDC, especially in the subgroup of DCC, as depression in the elderly may also be a prodromal state of AD. Compared to AD, FTD presented with both lower tau and lower p-tau levels in CSF. These findings are in accordance with the literature [2, 3, 17, 20].

### *A $\beta$ Peptide Patterns in PPA*

None of the investigated A $\beta$  peptides differed significantly between PPA and NDC. The major differences between PPA and AD were the decreased CSF levels of A $\beta_{1-42}$  and, subsequently, the ratios of A $\beta_{1-42}$ /A $\beta_{1-40}$  and A $\beta_{1-42}$ /A $\beta_{1-38}$  in the latter. Due to stable CSF A $\beta_{1-40}$  levels in both groups, paralleled by a tendency toward higher and lower CSF A $\beta_{1-38}$  levels in AD and PPA, respectively, the ratio of A $\beta_{1-38}$ /A $\beta_{1-40}$  differed between the two diagnostic groups.

When comparing the CSF A $\beta$  peptide patterns of PPA and FTD, the only difference was lower levels of A $\beta_{1-38}$  in the latter. This may somehow indicate a specificity of decreased CSF A $\beta_{1-38}$  levels in FTD as compared to other subgroups of the FTL spectrum. However, due to the lack of a confirmative neuropathological diagnosis, the pathophysiological implications of this finding remain to be elucidated in further investigations. Nevertheless, there is evidence of three distinct presenilin 1 mutations causing a clinical phenotype of FTD [21, 22], indicating a role of A $\beta$  peptide metabolism in the neurodegenerative process of FTD. Interestingly, presenilin mutations are also the most common cause of familial AD.

To the best of our knowledge, this is the first study on the three major CSF A $\beta$  peptides 1–40, 1–38 and 1–42 in the distinct group of PPA. A multiple case evaluation of patients with PPA (n = 3) reported CSF A $\beta_{1-42}$  to be in the normal range [23]. A subgroup evaluation of a study on the CSF biomarkers tau, p-tau and A $\beta_{1-42}$  in 34 patients with FTL in comparison to AD and control subjects found PPA to have the smallest overlap with AD-typical CSF biomarker patterns [24]. Despite large methodological differences between those studies, the results indicate A $\beta_{1-42}$  to be in the normal range in most patients with PPA, which is in line with our results. Although it missed

the level of significance, we noted a weak tendency toward lower CSF A $\beta_{1-42}$  levels in PPA than in NDC. This may be caused by overlapping AD pathology in PPA and may become more evident in a larger cohort of PPA patients. However, we cannot completely exclude the possibility that AD and PPA diagnoses have been mixed up. We did not find any previous report on CSF A $\beta_{1-38}$  and A $\beta_{1-40}$  in PPA.

### *Tau and p-Tau Proteins in PPA*

Neither tau nor p-tau was significantly altered between PPA and NDC. However, a previous multiple case report found slightly elevated tau levels in 3 cases of PPA [23]. In another study on biomarkers in FTL, a subgroup analysis revealed PPA to show the smallest overlap with AD-typical CSF biomarker patterns, indicating rather normal values for tau in the group of PPA [24]. Otherwise, tau was not able to differentiate PPA from AD in our study. Thus, CSF tau may somehow be a variable parameter in PPA, probably correlating more closely with the actual degree of neurodegeneration at the time of lumbar puncture than with the underlying disease entity. Interestingly, tau was significantly lower in FTD than in PPA, indicating a higher dynamic of neurodegeneration in the latter.

p-tau levels were also elevated in PPA, when compared to FTD, which may support the abovementioned hypothesis about possible dysfunction of phosphorylation regulatory mechanisms in FTD [20]. In comparison to AD, p-tau levels were significantly lower in PPA, underlining the differential diagnostic value of this parameter for AD differential diagnosis among dementias of other kind. Thus, hyperphosphorylation of tau remains a specific pathogenic core feature of AD in contrast to other dementias. Moreover, it seems that, due to a thorough clinical diagnostic workup, AD and PPA diagnoses have been mixed up only to a limited extend.

### *Limitations of the Study*

The reliance on clinical diagnosis limits the validity of our results because of potential misclassification. Another point of concern is that the methods used for measurement varied between A $\beta_{1-38}$  and A $\beta_{1-40}$  or A $\beta_{1-42}$ . However, an ELISA for A $\beta_{1-38}$  was not yet available at the time of measurement. Moreover, the analyses of p-tau, A $\beta_{1-38}$  and A $\beta_{1-40}$  were made with frozen samples, whereas A $\beta_{1-42}$  and total tau ELISA were conducted with unfrozen CSF samples.

## Conclusions

This is the first detailed report on biomarker patterns in PPA including the three most prominent A $\beta$  peptides in CSF (1–40, 1–38 and 1–42) in addition to the widely accepted AD CSF biomarkers tau and p-tau. The evaluation of these markers in PPA as compared to AD, FTD and NDC confirmed high p-tau levels and low levels of A $\beta$ <sub>1–42</sub>/A $\beta$ <sub>1–40</sub> and A $\beta$ <sub>1–42</sub>/A $\beta$ <sub>1–38</sub> as specific features of AD as compared to other dementias. Moreover, the finding of low CSF A $\beta$ <sub>1–38</sub> levels in FTD could be confirmed. Interestingly, PPA did not show this feature. In addition, PPA patients showed higher levels of CSF tau and p-tau than did FTD patients. Thus, our results show distinct CSF biomarker patterns for FTD and PPA. Further studies on larger patient groups will have to clarify the diagnostic value of this finding. Otherwise, a distinct clinical

presentation may be related to different kinds of neuropathology. Thus, the investigation of more homogeneous patient groups with neuropathologically confirmative diagnoses may display CSF biomarker changes even more precisely. This approach may also elucidate the pathophysiological implications of our results. We are convinced that these aspects deserve further investigation in appropriately designed studies.

## Acknowledgments

This study was supported by the following grants: cNEUPRO (EU contract No. LSHM-CT-2007-037950) and neuroTAS (EU contract No. LSHB-CT-2006-037953). The authors would like to thank Sabine Lehmann, Birgit Otte and Heike Zech for excellent technical assistance.

## References

- 1 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.
- 2 Sjögren M, Minthon L, Davidson P, Granerus AK, Clarberg A, Vanderstichele H, Vanmechelen E, Wallin A, Blennow K: CSF levels of tau,  $\beta$ -amyloid<sub>1–42</sub> and GAP-43 in frontotemporal dementia, other types of dementia and normal aging. *J Neural Transm* 2000;107:563–579.
- 3 Riemenschneider M, Wagenpfeil S, Diehl J, Lautenschlager N, Thöml T, Heldmann B, Drzezga A, Jahn T, Förstl H, Kurz A: Tau and A $\beta$ <sub>42</sub> protein in CSF of patients with frontotemporal degeneration. *Neurology* 2002;58:1622–1628.
- 4 Galasko D, Marder K: Picking away at frontotemporal dementia. *Neurology* 2000;58:1585–1586.
- 5 Pijnenburg YA, Schoonenboom SN, Mehta PD, Mehta SP, Mulder C, Veerhuis R, Blankenstein MA, Scheltens P: Decreased cerebrospinal fluid amyloid beta<sub>1–40</sub> levels in frontotemporal lobar degeneration. *J Neurol Neurosurg Psychiatry* 2007;78:735–737.
- 6 Andersen C, Jensen M, Lannfelt L, Lindau M, Wahlund LO: Amyloid A $\beta$ <sub>40</sub> CSF concentrations correlate to frontal lobe atrophy in frontotemporal dementia. *Neuroreport* 2000;11:287–290.
- 7 Bibl M, Mollenhauer B, Wolf S, Esselmann H, Lewczuk P, Kornhuber J, Wiltfang J: Reduced CSF carboxyterminally truncated A $\beta$  peptides in frontotemporal lobe degenerations. *J Neural Transm* 2007;114:621–628.
- 8 Bibl M, Mollenhauer B, Lewczuk P, Esselmann H, Wolf S, Trenkwalder C, Otto M, Stiens G, Rütger E, Kornhuber J, Wiltfang J: Validation of amyloid- $\beta$  peptides in CSF diagnosis of neurodegenerative dementias. *Mol Psychiatry* 2007;12:671–680.
- 9 Bibl M, Lewczuk P, Esselmann H, Mollenhauer B, Klafki HW, Welge V, Wolf S, Trenkwalder C, Otto M, Kornhuber J, Wiltfang J: CSF amyloid- $\beta$  1–38 and 1–42 in FTD and AD: biomarker performance critically depends on the detergent accessible fraction. *Proteomics Clin Appl* 2008;2:1548–1556.
- 10 Steinacker P, Hendrich C, Sperfeld AD, Jesse S, Lehnert S, Pabst A, von Arnim CA, Motaghay FM, Uttner I, Tumani H, Ludolph A, Otto M: Concentrations of  $\beta$ -amyloid precursor protein processing products in cerebrospinal fluid of patients with amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *J Neural Transm* 2009;116:1169–1178.
- 11 Steinacker P, Hendrich C, Sperfeld AD, Jesse S, von Arnim CA, Lehnert S, Pabst A, Uttner I, Tumani H, Lee VM, Trojanowski JQ, Kretschmar HA, Ludolph A, Neumann M, Otto M: TDP-43 in cerebrospinal fluid of patients with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Arch Neurol* 2008;65:1481–1487.
- 12 World Medical Organisation: Declaration of Helsinki. *BMJ* 1996;313:1448–1449.
- 13 McKhann G, Drachman D, Folstein M, Katzmann R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939–944.
- 14 Vanmechelen E, Vanderstichele H, Davidsson P, van Kerschaver E, van der Perre B, Sjögren M, Andreasen N, Blennow K: Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization. *Neurosci Lett* 2000;285:49–52.
- 15 Hulstaert F, Blennow K, Ivanoiu A, Schoonderwald HC, Riemenschneider M, de Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, Iqbal K, Pottel H, Vanmechelen E, Vanderstichele H: Improved discrimination of AD-patients using  $\beta$ -amyloid<sub>1–42</sub> and tau levels in CSF. *Neurology* 1999;52:1555–1562.
- 16 Wiltfang J, Esselmann H, Bibl M, Smirnov A, Otto M, Paul S, Schmid B, Klafki HW, Maler M, Dyrks T, Bienert M, Beyermann M, Rütger E, Kornhuber J: Highly conserved and disease-specific patterns of carboxyterminally truncated A $\beta$  peptides 1–37/38/39 in addition to 1–40/42 in Alzheimer's disease and patients with chronic neuroinflammation. *J Neurochem* 2002;81:481–496.
- 17 Blennow K: Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx* 2004;1:213–225.

- 18 Bibl M, Mollenhauer B, Esselmann H, Lewczuk P, Trenkwalder C, R  ther E, Kornhuber J, Otto M, Wiltfang J: CSF diagnosis of Alzheimer's disease and dementia with Lewy bodies. *J Neural Transm* 2006;114:621–628.
- 19 Bibl M, Mollenhauer B, Esselmann H, Schneider M, Lewczuk P, Welge V, Gross M, Falkai P, Kornhuber J, Wiltfang J: Cerebrospinal fluid neurochemical phenotypes in vascular dementias: original data and mini-review. *Dement Geriatr Cogn Disord* 2008; 25:256–265.
- 20 Sj  gren M, Davidsson P, Tullberg M, Mint-hon L, Wallin A, Wikkelso C, Gran  rus AK, Vanderstichele H, Vanmechelen E, Blennow K: Both total and phosphorylated tau are increased in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2001;70:624–630.
- 21 Deraut B, Kumar-Singh S, Engelborghs S, Theuns J, Rademakers R, Saerens J, Pickut BA, Peeters K, van den Broeck M, Vennekens K, Claes S, Cruts M, Cras P, Martin JJ, van Broeckhoven C, de Deyn PP: A novel presenilin 1 mutation associated with Pick's disease but not  $\beta$ -amyloid plaques. *Ann Neurol* 2004;55:617–626.
- 22 Zekanowski C, Golan MP, Krzysko KA, Lipczynska-Lojkowska W, Filipek S, Kowalska A, Rossa G, Peplonska B, Styczynska M, Maruszak A, Religa D, Wender M, Kulczycki J, Barcikowska M, Kuznicki J: Two novel presenilin 1 gene mutations connected with frontotemporal dementia-like clinical phenotype: genetic and bioinformatic assessment. *Exp Neurol* 2006;200:82–88.
- 23 Maeck L, Meller J, Otto M, Stiens G, Wiltfang J, Stoppe G: A $\beta$  peptide 1–42, tau protein and S-100B protein level in cerebrospinal fluid of three patients with primary progressive aphasia. *Neurosci Lett* 2002;333: 33–36.
- 24 Kapaki E, Paraskevas GP, Papageorgiou SG, Bonakis A, Kalfakis N, Zalonis I, Vassilopoulos D: Diagnostic value of CSF biomarker profile in frontotemporal lobar degeneration. *Alzheimer Dis Assoc Disord* 2008;22: 47–53.