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Reduced CSF carboxyterminally truncated Aβ peptides in frontotemporal lobe degenerations

M. Bibl[1], B. Mollenhauer[2], S. Wolf[2], H. Esselmann[3], P. Lewczuk[3], J. Kornhuber[3], J. Wiltfang[3]

[1] Department of Psychiatry, University of Goettingen, Goettingen, Germany
[2] Brigham and Women's Hospital, Center for Neurologic Diseases, Harvard Medical School, Boston, Massachusetts, USA
[3] Department of Psychiatry and Psychotherapy, University of Erlangen-Nuremberg, Erlangen, Germany

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Summary Cerebrospinal fluid (CSF) carboxyterminally truncated amyloid-beta (Aβ) peptides, Aβ1-42 and tau protein were evaluated in 30 patients with frontotemporal lobe degenerations (FTLD), 30 Alzheimer's disease (AD) patients and 30 non-demented disease controls (NDC) by Aβ-SDS-PAGE/immunoblot as well as commercial ELISAs for Aβ1-42 and total tau. FTLD displayed a significant drop of Aβ1-37 ($p = 2.7 \times 10^{-4}$), Aβ1-38 ($p = 4.2 \times 10^{-5}$) and Aβ1-42 ($p = 3.3 \times 10^{-4}$). Aβ1-42 was selectively decreased in AD ($p = 8.5 \times 10^{-10}$). Decreased Aβ1-38 enabled contrasts of beyond 85% to distinguish FTLD from AD and NDC patients, alone or in combination. Accordingly, low CSF Aβ1-37 and Aβ1-38 represent a biomarker candidate for FTLD and may reflect disease-specific changes of APP metabolism. Further validation should be carried out on dementias other than AD, diagnostically relevant control groups without dementia and without any evident affection of the central nervous system and subgroups of FTLD. Moreover, independent methods of measurement should be applied to CSF Aβ1-38.

Keywords: Alzheimer's disease, frontotemporal degeneration, cerebrospinal fluid, amyloid-β peptides, biomarkers

Abbrevations: *Aβ peptides* Amyloid-beta peptides, *Aβ-SDS-PAGE/immunoblot* amyloid-beta-sodium-dodecyl-sulphate-polyacrylamide-gel-electrophoresis with western immunoblot, *AD* Alzheimer's disease, *APP* beta-amyloid precursor protein, *bicine N,N′*-bis-[2-hydroxyethyl]glycine, *%C* percentage of *N,N′*-bis-acrylamide, *CCD-camera* charge coupled device camera, *CSF* cerebrospinal fluid, *Ct-elongated* carboxyterminally elongated, *Ct-truncated* carboxyterminally truncated, *ECL* enhanced chemiluminescence, *ELISA* Enzyme Linked Immunosorbent Assay, *FTLD* frontotemporal lobe degenerations, *MMSE* Mini-Mental-Status Examination, *NDC* non-demented disease controls, *NINCDS-ADRDA* National Institute Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association, *SDS* sodium dodecyl sulphate, *%T* percentage of acrylamide

Correspondence: Professor Dr. Jens Wiltfang, Molecular Neurobiology Lab, Department of Psychiatry and Psychotherapy, University of Erlangen-Nuremberg, Schwabachanlage 6, 91054 Erlangen, Germany
e-mail: Jens.Wiltfang@psych.imed.uni-erlangen.de;
Dr. Mirko Bibl, Neurobiology Lab, Department of Psychiatry and Psychotherapy, University of Goettingen, von-Siebold-Str. 5, 37075 Göttingen, Germany
e-mail: mbibl@gwdg.de

Introduction

The heterogeneous group of frontotemporal lobe degenerations (FTLD) and Alzheimer's disease (AD) share neurochemical and a set of clinical features, although temporal appearance of symptoms during the course of disease and neuropathological findings differ remarkably between the two dementia syndromes.

The extracellular deposition of aggregated amyloid-beta (Aβ) peptides, mainly Aβ1-42, as amyloid-plaques is a neuropathological hallmark of Alzheimer's disease (AD) (Glenner and Wong, 1984), whereas amyloid plaques are rarely found in FTLD patients (Arnold et al., 2000). Aβ1-42-levels in CSF are significantly reduced in Alzheimer's disease and to a lesser degree in FTLD (Hulstaert et al., 1999).

Aβ peptides derive from a transmembrane amyloid precursor protein (APP), when cleaved by two enzymes, β- and γ-secretase (Haas and Selkoe, 1993). Distinct γ-secretase activities are hypothesized to be responsible for generation of either carboxyterminally truncated (Ct-truncated) or elongated (Ct-elongated) Aβ peptides as referenced to Aβ1-40 (Citron et al., 1996). Thus, the measurement of the different originating Aβ peptide species in CSF can be assumed to more adequately represent disease-specific changes of APP metabolism rather than the simple absolute Aβ1-42 levels (Wiltfang et al., 2001; Bibl et al., 2006a, b).

This prompted us to determine the Ct-truncated Aβ peptides 1-37, 1-38 and 1-39 in addition to 1-40 and 1-42 (Aβ peptide pattern) in the CSF of FTLD, AD and non-demented disease controls by the recently established Aβ-SDS-PAGE/immunoblot.

Three sets of clinical criteria for the differential diagnosis of frontotemporal degenerations have been published so far. The latest set of criteria has been suggested by McKhann et al. (2001), mainly to aid routine clinical diagnosis. Otherwise, the clinical criteria of Neary et al. (1998) are most helpful when focussing on the three prototypic subtypes of FTLD, frontotemporal dementia (FTD), primary progressive aphasia (PPA) and semantic dementia (SD). Nevertheless, clinical diagnosis often remains crucial in a definite case and biomarkers for applicable diagnostic testing are still under intensive investigation. The constellation of reduced Aβ1-42 in CSF accompanied by an elevation of tau protein can be found in both FTD and AD (Blennow, 2004). Inconsistent data about the performance of these proteins as an applicable biomarker for the differential diagnosis of FTD and AD have been published to date (Sjögren et al., 2000a; Riemenschneider et al., 2002). This may be due to the fact that the diagnosis of the above studies referred to different sets of clinical criteria and selected different non-demented control groups for comparison.

The present study addressed the question, of whether the Aβ peptide metabolism of FTLD would be differentially altered as compared to clinically relevant differential diagnoses, such as AD, depression and other non-dementive disorders. Moreover, we evaluated the power of Aβ peptide patterns, Aβ1-42 and tau protein to validate the clinical diagnosis of FTLD.

Patients and methods

Aβ peptide patterns were analysed by Aβ-SDS-PAGE/immunoblot. Aβ1-42 and total tau protein were determined by ELISA in the CSF of patients with FTLD, AD and non-demented disease controls (NDC). A total of 90 patients, whose CSF samples had been referred to our laboratory for neurochemical analysis between 2000 and 2004, were divided into 3 diagnostic groups according to their clinical diagnosis. The patients were selected on wards and the dementia outpatient clinic of the Universitiy of Goettingen. Five AD patients came from the dementia outpatient clinic of the Universitiy of Erlangen. Both specialized centres followed a standardized protocol of sample handling. All neurochemical measurements and quantifications were performed in the laboratory of neurobiology of the University of Goettingen by two very experienced technical assistants blinded to clinical diagnosis. Diagnosis was established by a neurologist, a psychiatrist and a neuropsychologist, all highly experienced in clinical differential diagnosis of dementias, on the basis of thorough anamnesis, clinical examination, results of neuropsychological assessment, clinical records of the patients and the best clinical judgement. All three investigators were blinded to the neurochemical outcome measures. Investigations were carried out with the informed consent of all patients or, for patients with severe dementia, their next of kin. If possible, neuropsychological assessment (MMSE or SKT at minimum) was performed on patients suffering from cognitive impairments at the time of lumbar puncture.

The study was conducted under the guidelines of the Declaration of Helsinki (World Medical Organisation, 1996) and approved by the ethics committee of the University of Goettingen and Erlangen-Nuremberg.

Patients with frontotemporal degenerations

All 30 patients (21 men and 9 women) of this group fulfilled the DSM IV and the latest consensus criteria for FTLD (McKhann et al., 2001). The consensus criteria of Neary et al. (1998) were applied to the clinical diagnosis of FTD (n = 24), PPA (n = 5) and semantic dementia (SD, n = 1), respectively. Neuropsychological testing, including Mini-Mental-Status-Evaluation (MMSE), clock drawing, CERAD and more detailed examination by experienced neuropsychologists was carried out on 24 patients. MMSE was available for 26 patients, the mean MMSE score was 20.7 ± 8.9 (mean ± SD). Any neuropsychological assessment was hindered in 4 patients by severe lingual or aphasic deficits. Age of this group was 61.6 ± 11.5 years (mean ± SD).

All enrolled patients received computerized tomography (CT) or magnetic resonance imaging (MRI) of the brain. Functional imaging using either 99mTc-hexamethylpropyleneamine oxime single photon emission computerised tomography (SPECT) or [18F]fluorodeoxyglucose PET investigation of the regional cerebral blood flow was available for 22 patients. Included patients exhibited frontal, frontotemporal or anterior temporal focal atrophy or marked hypometabolism. Aβ1-42 ELISA was available for 22 and tau ELISA for 25 patients.

Patients with Alzheimer's disease

All 30 patients (13 men and 17 women) of this group fulfilled the DSM IV criteria for AD and the NINCDS-ADRDA criteria for clinical diagnosis of probable AD (McKhann et al., 1984). Age of this group was 65.4 ± 7.3 years (mean ± SD). Neuropsychological testing, including MMSE, clock drawing, CERAD and more detailed examination by experienced neuropsychologists was carried out on 29 patients. MMSE was not possible in one patient due to severe cognitive deficits. The mean MMSE score was 19.3 ± 5.4 (mean ± SD) in this group. Patients received structural CT or MRI brain imaging. Included patients exhibited global cortical atrophy or temporal, parietotemporal, frontotemporal focal atrophy or marked hypometabolism of these regions. The result of brain imaging was not available in three patients. Aβ1-42 ELISA and tau ELISA were available for 26 patients.

Non-demented disease controls (NDC)

This group consisted of 30 non-demented patients (10 men and 20 women), who underwent lumbar puncture for the differential diagnosis of dementia or other differential diagnostic reasons. Patients with persistent cognitive decline for more than six month an MMSE score below 26 or clear focal atrophy in brain imaging (CT or MRI) were excluded.

Age of this group was 61.5 ± 11.1 years (mean ± SD). The group included patients suffering from depression (n = 16), panic attacks with vertigo (n = 1), peripheral facial nerve palsy, borreliosis without central manifestation (n = 1), facial hemi spasm (n = 1), antiphospholipidsyndrome (n = 1), paraneoplastic syndrome (n = 2), schizophrenia (n = 1), polymorphic psychosis (n = 1), mania (n = 3), bipolar affective disorder (n = 1), motoneuron disease without dementia (n = 1), insomnia (n = 1). All patients with cognitive complaints (n = 22) were assessed by MMSE at minimum. The mean MMSE score was 28.5 ± 1.5 (mean ± SD). The cognitive impairments of all depressive patients improved after antidepressant medica-

tion. Brain imaging (CT or MRI) were available for 21 patients. Aβ1-42 ELISA and tau ELISA was available for 23 patients.

Preanalytical treatment of CSF for Aβ-SDS-PAGE immunoblot, Aβ1-42 and tau ELISA

The preanalytical handling of all included CSF samples followed a standardized protocol according to previously published data (Bibl et al., 2004).

CSF was drawn from patients by lumbar puncture sampled in polypropylene vials and centrifuged (1000 *g*, 10 min, 4°C). Aliquots of 200 µl were kept at room temperature for a maximum 24 h before storage at −80°C for subsequent Aβ-SDS-PAGE/immunoblot analysis. There was no impact of storage time before freezing as controlled for up to 48 h on Aβ peptide or tau protein levels. Freezing of samples was conducted by directly cooling 200 µl of CSF in polypropylene cups down to −80°C without an intermediate temperature stage. Shock freezing was not performed. The samples did not undergo additional freeze and thaw cycles. CSF for Aβ1-42- and tau ELISA analysis was stored at +4°C and analyzed within two days.

Aβ-SDS-PAGE/immunoblot

For separation of Aβ peptides and subsequent detection, 10 µl of unconcentrated CSF were boiled in a sample buffer for SDS-PAGE, and Aβ-SDS-PAGE/immunoblot was conducted as published elsewhere (Wiltfang et al., 2002; Bibl et al., 2004).

CSF samples of each individual patient were run as triplicates and each gel carried a four step dilution series of the synthetic Aβ peptides Aβ1-37, Aβ1-38, Aβ1-39, Aβ1-40 and Aβ1-42. Synthetic peptides Aβ1-38, Aβ1-40, Aβ1-42 were obtained from Bachem (Bubendorf, Switzerland), Aβ1-37 and Aβ1-39 were synthesized automatically according to Janek et al. (2001). Synthetic Aβ peptide dilution series were created as described previously (Bibl et al., 2004). In detail, the stock solution of synthetic Aβ peptides (0.5 mg/mL) was diluted to 24 ng/mL (Aβ1-40), 12 ng/mL (Aβ1-38 and Aβ1-42), and 6 ng/mL (Aβ1-37 and Aβ1-39). The peptides were mixed to create the first dilution step of synthetic Aβ peptide dilution series and the mixture was diluted 1:2 three more times.

The amino-terminal-selective mouse monoclonal antibody 1E8 (Schering, Germany) was applied overnight at 4°C for detection of the antigen. Further incubation with a biotinylated anti-mouse polyclonal antibody (Vector Laboratories, Burlingame, USA) and horseradish peroxidase coupled streptavidin (Amersham Pharmacia Biotech, England) for was performed for 1 h each. Washing steps were performed in between. Enhanced Chemiluminescence (ECL) served for visualization and bands were quantified from individual blots of each patient relative to standard Aβ peptide dilution series using a charge coupled device camera (CCD-camera).

The detection sensitivity for the 1E8 in this optimized immunoblot procedure was 0.6 pg (Aβ1-38, Aβ1-40) and 1 pg (Aβ1-37, Aβ1-39, Aβ1-42), respectively (Bibl et al., 2004). The inter- and intra-assay coefficients of variation for 80 as well as for 20 pg of synthetic Aβ peptides were below 10% (Wiltfang et al., 2002; Bibl et al., 2004).

Tau protein ELISA and Aβ1-42 ELISA

The commercially available assays Innotest hTAU Antigen and Innotest β-Amyloid$_{(1-42)}$, Innogenetics (Ghent, Belgium) were applied for the quantification of tau protein and Aβ1-42 levels in CSF, respectively. Tau and Aβ1-42 ELISA were performed according to previously published standard methods (Hulstaert et al., 1999).The inter- and intra-assay variability of the Aβ1-42-ELISA was below 10%, the detection sensitivity for Aβ1-42 was 50 pg/ml (Hulstaert et al., 1999).

Statistical analysis

Aβ peptide and tau levels were expressed as absolute values (ng/ml). The data on peptide levels have been obtained from individual blots of each individual patient. We have characterized patient groups by mean and standard deviation (SD). The Mann-Whitney U-test was applied to evaluate significant group differences. The Wilcoxon signed ranks test was used to calculate significant differences of two related samples. Multiple comparisons were not performed.

The two-sided level of significance was taken as $p < 0.05$. A p-value less than 0.01 was considered as highly significant. Receiver operating characteristic (ROC) curve analysis was used to determine cut-off points. Additionally, the optimal cut-off level for dichotomising values was selected as the situation maximizing the Youden index. Only the best discriminating cut-off values alone or in combination are presented. Computations were performed using the statistical software package SPSS, version 10.0.

Results

The mean age did not significantly differ between the diagnostic groups ($p > 0.05$).

The mean MMSE score did not significantly differ between FTLD and AD ($p > 0.05$) and was significantly higher for the NDC group ($p = 1.5 \times 10^{-7}$ and 1.5×10^{-6}, respectively).

The gender distribution was imbalanced among the diagnostic groups. However, a correlation of any of the investigated Aβ peptides with gender was not found.

Aβ-peptide patterns in the Aβ-SDS-PAGE/immunoblot

In the presence of urea, the Aβ-SDS-PAGE/immunoblot allowed the electrophoretic separation and subsequent analysis of Aβ1-37, Aβ1-38, Aβ1-39 and Aβ1-40 in addition to Aβ1-42. All Aβ peptides migrate as a single band, if urea is absent in otherwise unchanged separation gels (Fig. 1).

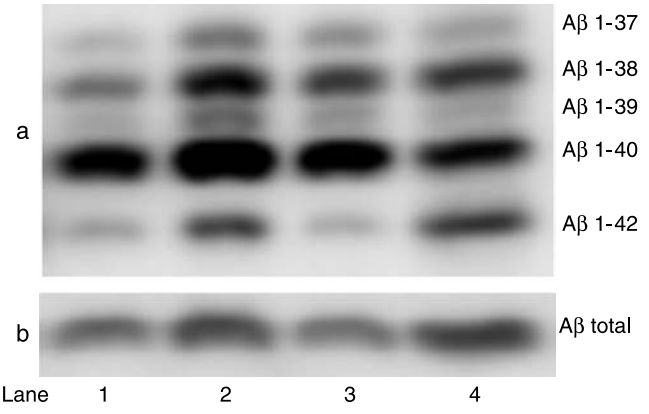

Fig. 1. Urea-based Aβ-SDS-PAGE/immunoblot (**a**) and conventional SDS-PAGE (**b**) of CSF (*1–3*) and synthetic Aβ peptides 1-37, 1-38, 1-39, 1-40, 1-42 (*4*). Ten microliters of an unconcentrated CSF have been applied to each lane. The figure shows a blot of CSF of a patient with FTD (*2*), NDC (*3*) and AD (*4*), respectively

The total amount of Aβ peptides did not significantly differ among the diagnostic groups ($p > 0.05$). Both dementia groups, FTLD and AD displayed decreased absolute levels of Aβ1-42 relative to the NDC group as measured by the Aβ-SDS-PAGE/immunoblot, however, this drop was much more pronounced in AD ($p = 3.3 \times 10^{-4}$ and $p = 8.5 \times 10^{-10}$, respectively). In contrast, the Ct-truncated Aβ peptides Aβ1-37 and Aβ1-38 were selectively reduced in FTLD as compared to NDC ($p = 2.7 \times 10^{-4}$ and $p = 4.2 \times 10^{-5}$) and AD ($p = 2.5 \times 10^{-3}$ and $p = 1.2 \times 10^{-3}$), respectively. Aβ1-40 was slightly elevated in FTLD and AD, whereas Aβ1-39 was found to be increased only in AD and decreased in FTLD. None of these alterations were significant to the level of $p = 0.05$. Otherwise, the percentage concentration of Aβ1-40 relative to the sum of all measured Aβ peptides (Aβ1-40%) was significantly increased in FTLD ($p = 2.2 \times 10^{-7}$). The absolute and percentage abundances of Aβ peptides of each diagnostic group are summarized in Table 1.

Table 1. *Aβ peptide patterns, Aβ1-42 and tau in the CSF of the diagnostic groups*

Diagnosis	NDC (n = 30)		AD (n = 30)		FTLD (n = 30)	
	MW	±SD	MW	±SD	MW	±SD
Age	61.5	11.09	65.43	7.29	61.6	11.48
MMSE	28.5	1.47	19.31	5.44	20.37	8.96
Tau ELISA	0.23	0.13	0.73	0.41	0.32	0.26
Aβ1-42 ELISA	0.86	0.28	0.37	0.13	0.70	0.20
Aβ1-42/tau[1]	4.82	2.81	0.65	0.34	3.49	1.81
Aβ1-37 (ng/ml)	1.36	0.49	1.21	0.47	0.90	0.42
Aβ1-38 (ng/ml)	2.26	0.60	2.17	0.81	1.60	0.72
Aβ1-39 (ng/ml)	1.27	0.38	1.28	0.44	1.17	0.46
Aβ1-40 (ng/ml)	8.42	1.98	8.27	2.81	8.67	2.43
Aβ1-42 (ng/ml)	1.89	0.72	0.60	0.23	1.23	0.58
total Aβ (ng/ml)[2]	15.32	3.55	13.68	4.45	13.67	4.25
Aβ1-37%[3]	8.71	1.88	8.80	1.88	6.47	1.49
Aβ1-38%[3]	14.79	2.09	15.74	2.09	11.49	2.09
Aβ1-39%[3]	8.23	1.39	9.35	1.63	8.41	1.53
Aβ1-40%[3]	55.36	6.28	60.58	5.18	64.04	4.45
Aβ1-42%[3]	12.16	3.48	4.46	1.23	8.79	2.26

[1] Ratio of absolute Aβ1-42 and tau levels as measured by ELISA; [2] total Aβ peptide concentration as measured by the sum of all investigated Aβ peptides; [3] percentage abundance of Aβ peptides relative to the sum of all investigated Aβ peptides.

Decreased levels of Aβ1-38 in FTLD allowed a distinction from AD with a sensitivity and specificity of 77 and 67%, respectively. The less pronounced decrease of Aβ1-42 in FTLD enabled discrimination from AD with a sensitivity of 87% and a specificity of 70%. Among all investigated peptides, these two peptides displayed the highest accuracy as a single marker to differentiate between FTLD and AD. This prompted us to investigate the combination of both peptides for its diagnostic accuracy. At a specificity of 97%, the ratio of Aβ1-42 to Aβ1-38 exhibited a sensitivity of 93% for detection of FTLD among AD patients.

Due to similar alterations of the two peptides in FTLD as compared to NDC, the Aβ1-42/Aβ1-38 ratio showed no reasonable accuracy to detect FTLD among NDC or in a combined group of AD and NDC.

With a sensitivity of 77% and a specificity of 73%, the reduction of Aβ1-38 in FTLD reached the maximum diagnostic accuracy among the absolute values of each single Aβ peptide to differentiate FTLD from NDC.

Taken together with the slightly elevated Aβ1-40 levels in FTLD, the ratio of Aβ1-38 to Aβ1-40 improved the discrimination of FTLD and NDC to a sensitivity of 87% and a specificity of 90%. Additionally, this ratio provided a sensitivity of 87% and a specificity of 87% for detection of FTLD among AD patients.

Moreover, the ratio of Aβ1-38 to Aβ1-40 achieved the best discrimination of FTLD among both NDC and AD, yielding a sensitivity and specificity of 87 and 88%, respectively.

The cut-off points, sensitivities and specificities for each differential diagnostic testing are summarized in Table 2.

ELISA's for tau protein and Aβ1-42

Tau protein was significantly elevated in AD ($p = 3.6 \times 10^{-7}$), but only marginally in FTLD ($p > 0.05$), as

Table 2. *Cut-off points, sensitivities and specificities for each differential diagnostic testing*

Differential diagnosis	Parameter	Cut-off	Sensitivity (%)	Specificity (%)	Youden index
AD versus NDC	ELISA Aβ1-42/tau	1.383	100	96	1.96
	Aβ1-42/Aβ1-38	0.475	100	97	1.97
FTLD versus NDC	ELISA Aβ1-42/tau	4.188	81	61	1.42
	Aβ1-38/Aβ1-40	0.215	87	90	1.77
FTLD versus AD	ELISA Aβ1-42/tau	1.612	86	100	1.86
	Aβ1-38/Aβ1-40	0.215	87	87	1.74
FTLD versus NDC and AD	ELISA Aβ1-42/tau	0.978	100	45	1.45
	Aβ1-38/Aβ1-40	0.215	87	88	1.75

compared to NDC. This assay reached a sensitivity of 91% and a specificity of 81% for the discrimination of FTLD and AD. The sensitivity and specificity of FTLD detection among NDC was 50 and 61%, respectively.

FTLD and AD presented with decreased absolute levels of Aβ1-42 compared to the NDC group as measured by ELISA ($p = 3 \times 10^{-3}$ and 9.7×10^{-8}, respectively). Absolute Aβ1-42 levels discriminated FTLD and AD with a sensitivity of 91% and a specificity of 89%. FTLD could be distinguished from NDC with a sensitivity of 68% and a specificity of 70%.

By combining Aβ1-42 and tau protein levels to a ratio of Aβ1-42/tau, the diagnostic power of the test for FTLD detection among AD was increased to a sensitivity of 86% and a specificity of 100%. The sensitivity and specificity for detection of FTLD among NDC patients was 81 and 61%, respectively. The detection of FTLD among both NDC and AD yielded a sensitivity of 100% and specificity of 45%.

The absolute values for Aβ1-42 and tau as measured by ELISA and the cut off points, sensitivities, specificities and maximum Youden index for each differential diagnostic testing are summarized in Tables 1 and 2, respectively.

Aβ1-42 levels in dependence of the method of measurement

Aβ1-42 was comparatively measured by commercially available ELISA and the Aβ-SDS-PAGE/immunoblot. The comparison of values revealed higher levels of Aβ1-42 as determined by the Aβ-SDS-PAGE/immunoblot. In a total of 71 comparatively measured patients this was highly

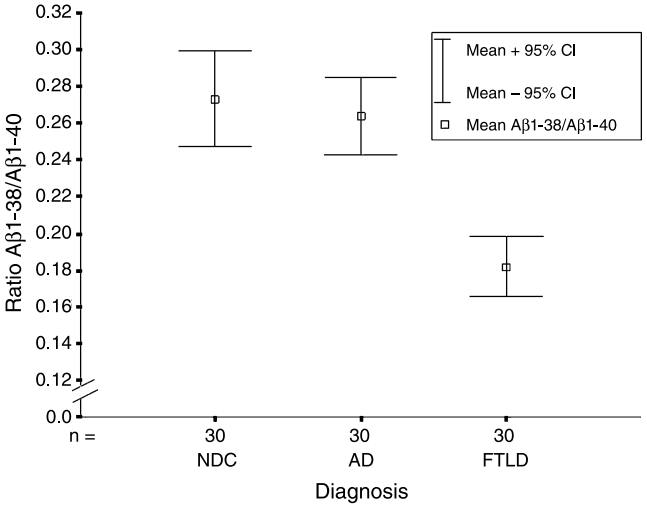

Fig. 2. Mean and 95% confidence interval (*CI*) of Aβ1-38/Aβ1-40 for each diagnostic group

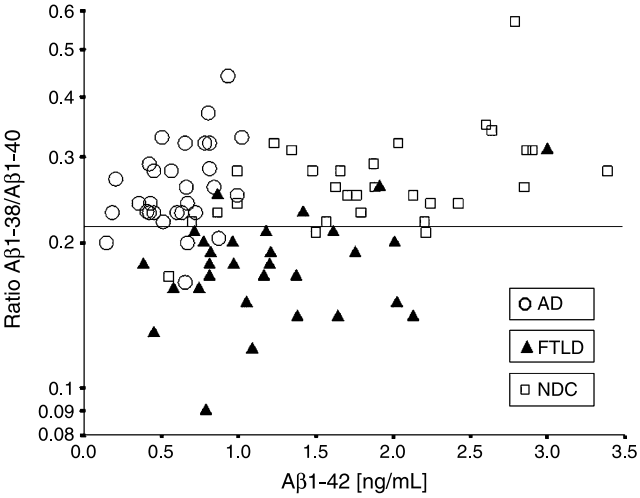

Fig. 3. Scatterplot of FTLD, AD and NDC patients divided by the ratio of Aβ1-38/Aβ1-40 and Aβ1-42 [ng/mL]. The best cut-off (0.215) for differentiating FTLD among AD and NDC using Aβ1-38/Aβ1-40 is indicated by a permanent line within the figure

significant ($p = 2.8 \times 10^{-12}$). Otherwise the degree of difference of values as measured by ELISA and the Aβ-SDS-PAGE/immunoblot depended on the diagnostic group. The levels of significance were found to be as follows: $p = 4.6 \times 10^{-6}$ for the NDC group ($n = 23$), 2.1×10^{-5} for AD ($n = 26$) and 1.2×10^{-4} for FTLD.

Discussion

CSF samples of 90 patients suffering from FTLD, AD and variuos neuropsychiatric diseases without dementia were investigated by commercially available ELISAs for Aβ1-42, tau protein and the recently established quantitative Aβ-SDS-PAGE/immunoblot (Wiltfang et al., 2002; Bibl et al., 2004) for disease-specific Aβ peptide patterns and tau levels.

The Aβ-SDS-PAGE/immunoblot revealed a pattern of the Ct-truncated Aβ peptides 1-37, 1-38, 1-39 in addition to 1-40 and 1-42 and demonstrated that the reduction of Aβ1-42 in AD was selective and not paralleled by a significant decrease of any of the other Aβ peptide species. In contrast, FTLD patients exhibited a less pronounced drop of Aβ1-42 that was paralleled by reduced levels of Aβ1-37 and Aβ1-38.

Aβ1-42 in FTLD and AD

Despite the fact that amyloid plaques are not a common neuropathological feature in FTLD, patients exhibit reduced levels of Aβ1-42 in CSF, a phenomenon that has also been recently described for Creutzfeld-Jacob's disease

(Wiltfang et al., 2003). This has been explained by an SDS-stable binding of the peptide to a putative carrier that masks its epitopes to antibodies during immunologic detection (e.g. immunoblot procedures or ELISA) (Wiltfang et al., 2003). The same mechanism may be suggested for FTLD and fits in with the finding that the Aβ-SDS-PAGE/immunoblot demonstrated a more pronuonced reduction of Aβ1-42 in FTLD and AD as compared to NDC than the ELISA. Moreover, the Aβ-SDS-PAGE/immunoblot yielded significantly higher absolute Aβ1-42 levels than the ELISA. These data indicate that the peptide could be partly stripped off its binding partner during SDS denaturation and the portion of SDS-stable bindings of the peptide to its putative carrier may be increased in both neurodegenerative diseases. Whilst the identity of this carrier remains obscure to date, apolipoproteins (Koudinov et al., 1996), formation of SDS-stable oligomers (Podlisny et al., 1995), supramolecular aggregates of Aβ peptides (Pitschke et al., 1998) have been reported as candidate binding partners for Aβ peptides in CSF. Accordingly, we hypothesize the Aβ peptide metabolism to be involved into the pathogenesis of FTLD, which by no means the mandatory formation of amyloid plaques. This is underlined by a recent report of presenilin1 mutation that is associated with Pick-type tauopathy, but not amyloid plaques (Dermaut et al., 2004). However, we cannot exclude overlapping AD pathology causing the reduced Aβ1-42 values in our FTLD patients, but the distinctiveness of the evaluated peptide patterns, especially with regard to Aβ1-37 and Aβ1-38 do not argue in favour of this hypothesis. Moreover, our data on tau and Aβ1-42 in FTD and AD reconfirm a recent autopsy controlled study of others (Grossman et al., 2005).

Ct-truncated Aβ peptides 1-37 and 1-38 in FTLD

To the best of our knowledge, Aβ peptides other than Aβ1-42 have not been investigated systematically in FTLD to date. One report suggested a negative correlation of CSF Aβ1-40 levels and frontal lobe atrophy in FTD (Andersen et al., 2000). Although this study lacks a nondemented control group and did not investigate AD patients for further comparison, their findings indicate that carboxyterminally shorter forms of Aβ peptides (i.e. shorter than Aβ1-42) play a special role in the pathogenesis of FTD. Taken together with our results, carboxyterminally shortened Aβ peptides seem to be more directly involved in pathologic events in FTLD, rather than in AD. We can only speculate that the reduction of the Ct-truncated Aβ peptides 1-37 and 1-38 in FTLD might be due to similar mechanisms like the reduction of Aβ1-42. One explanation could be that the aforementioned carrier, given its existence, exhibits a similar affinity to carboxyterminally shortened and elongated Aβ peptides, but leaves out the middle-length Aβ peptide species (Aβ1-40 and Aβ1-39) in FTLD. Otherwise, an overall reduction of Aβ peptides, as has been reported for CJD (Wiltfang et al., 2003), could be counteracted by a selective up regulation of Aβ1-40 and Aβ1-39 that is probably related to marked frontal lobe atrophy in FTLD (Andersen et al., 2000). This hypothesis is strengthened by our present finding of significantly elevated Aβ1-40 levels relative to the sum of all investigated Aβ peptides (Aβ1-40%) in FTLD as compared to NDC and AD. Absolute Aβ1-40 levels remained virtually unchanged among the diagnostic groups and the total amount of all measured Aβ peptides tended to be lower in FTLD as compared to NDC. However, both mechanisms could aggravate each other and lead to a disease-specific disturbance of Aβ peptide homeostasis. We have previously reported the selective elevation of Aβ1-38 in chronic inflammatory diseases (Wiltfang et al., 2002). Given the reduction of Aβ1-38 in FTLD in this study, the results moreover indicate a minor role of inflammatory processes in neurodegenerative mechanism of FTLD.

Diagnostic value of Aβ1-42 and tau ELISA for FTLD

In the ELISA, FTLD patients herein presented with a significant drop of Aβ1-42 and a slight elevation of tau levels. Aβ1-42 levels are claimed to show a reduction in FTLD and FTD, respectively, that is less marked than in AD and absolute values are reported to range between controls and AD (Hulstaert et al., 1999; Sjögren et al., 2000a; Riemenschneider et al., 2002). Tau values are often found to be normal (Hulstaert et al., 1999; Sjögren et al., 2000a, b, 2001) or mildly elevated (Blennow et al., 1995; Riemenschneider et al., 2002) in FTLD and FTD, respectively, as compared to non-demented controls, but also higher levels with a considerable overlap of value with AD have been reported (Green et al., 1999). In line with previous studies of others (Riemenschneider et al., 2002), we could discriminate AD from FTLD and FTD, respectively, at a highly significant level by a more prominent reduction of Aβ1-42 and a striking elevation of tau levels in the CSF of AD patients. With a sensitivity and specificity of 88 and 100%, respectively, we found the diagnostic accuracy of the combined evaluation of Aβ1-42 and tau to be sufficient for the differentiation of both dementias. Otherwise, the detection of FTLD among non-demented disease controls with 80% sensitivity and 64% specificity was disappointing and did not fulfil the requirements for an applicable diagnostic test.

The current literature provides only one study that reported an acceptable diagnostic accuracy of these biomarkers alone or in combination to discriminate FTLD from both AD and non-demented controls (Riemenschneider et al., 2002). The better discrimination of FTLD from non-demented controls in the aforementioned study particularly may be due to the selection of control subjects, as the control group comprised patients with lumboischialgia without any evident affection of the CNS. In contrast, we investigated neuropsychiatric disease controls that can be considered as more relevant for clinical differential diagnosis of FTLD in most cases. Otherwise, both studies reveal a similar diagnostic accuracy of the test for detection of AD patients among non-demented patients and FTLD, respectively. Nevertheless, the pattern of mildly increased tau and moderately decreased Aβ1-42 is not unique for FTD (Galasko and Marder, 2002) and also can be found in other dementias, such as vascular dementia (Hulstaert et al., 1999; Andreasen et al., 2001) or dementia with Lewy bodies (Mollenhauer et al., 2005). Thus, it can be assumed that the diagnostic value of these biomarkers to discrminate FTD among other dementias than AD will be limited (Galasko and Marder, 2002).

Diagnostic value of Aβ peptide patterns for FTLD

The Aβ-SDS-PAGE/immunoblot yielded a considerably higher accuracy in differentiating FTLD from neuropsychiatric disorders without dementia than the combined Aβ1-42/tau assay. Moreover, there is currently no evidence for a slective drop of Ct-truncated Aβ peptides in dementias other than FTLD. The introduction of ratios of the differentially altered Aβ peptide species relative to each other improved the diagnostic test accuracy for each differential diagnostic question. First, this may be due to disease-specific interactions of each ongoing neurodegenerative dementia process with APP metabolism, which cannot be adequately represented by the sole measurement of absolute Aβ1-42 levels (Wiltfang et al., 2001). It remains an open question, whether these alterations of APP metabolism represent a causal event in the pathogenesis of neurodegeneration or just an epiphenomenon. Second, the percentage abundance of each Aβ peptide species displayed a lower interindividual variance of values, than its absolute levels (Wiltfang et al., 2003). This may be explained by the assumption that the abundances of single Aβ peptide species are closely correlated to each other and are thus regulated in narrow limits, whereas the total amount of Aβ peptides varies interindividually (Wiltfang et al., 2002, 2003). Using these ratios, the evaluation of CSF Aβ peptides meets the criteria recommendations of an international consensus group for applicable biological markers of dementia (Wiltfang et al., 2005) for a suitable diagnostic test. In addition, these distinct alterations of CSF Aβ peptide patterns in FTLD and AD, respectively, indicate disease-specific changes of APP metabolism during neurodegenerative process in each of the two dementias.

Limitations of the study

We are aware of the fact that the power of the presented data is limited by the reliance on clinical diagnosis, which is claimed to misclassify 15–20% of dementia cases (Verbeek et al., 2003). Thus, the value of these promising data with regard to their pathophysiological and differential diagnostic meaning will have to be further elucidated in larger and neuropathologically controlled studies.

Further limitations arise from the clinical heterogeneity of FTLD and the study size, especially for PPA. Likewise, dementias other than AD will have to be systematically evaluated for comparison to more adequately estimate the value of the presented data for a routine diagnostic test. Moreover, we currently employ ELISA and mass spectrometric methods to reconfirm our results with independent methods.

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