No association of CSF biomarkers with APOEε4, plaque and tangle burden in definite Alzheimer’s disease

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The CSF biomarkers β-amyloid peptide (Aβ1–42), total tau protein (T-tau) and tau phosphorylated at threonine 181 (P-tau181P) were determined in autopsy-confirmed Alzheimer’s disease patients in order to study possible associations with the ε4 allele of APOE and density and spread of plaques (SP) and tangles (NFT).

CSF levels of Aβ1–42, T-tau and P-tau181P were determined in 50 Alzheimer’s disease patients using commercially available single parameter ELISA kits (INNOTEST®). Genomic DNA was extracted from whole blood and the APOE genotype was determined using standard methods. Tangle burden was assessed by means of Braak’s NFT stages (I–VI), whereas the plaque burden was assessed by means of Braak’s SP stages (A–C).

CSF biomarker levels were not different when comparing ε4 carriers (n=21) and non-carriers (n=29) (P>0.05 for all comparisons). No significant correlations between the number of ε4 alleles (0, 1 or 2) and CSF levels of Aβ1–42 (Spearman Rank Order: r = −0.057, P = 0.695), T-tau (r = 0.104, P = 0.472) and P-tau181P (r = 0.062, P = 0.668) were found. Braak’s SP (Aβ1–42: r = −0.155, P = 0.280; T-tau: r = −0.044, P = 0.763; P-tau181P: r = −0.010, P = 0.947) and NFT (Aβ1–42: r = −0.145, P = 0.315; T-tau: r = 0.117, P = 0.415; P-tau181P: r = 0.150, P = 0.296) stages were not significantly correlated with CSF biomarker levels.

In conclusion, CSF levels of Aβ1–42, T-tau and P-tau181P were not associated with ε4, tangle or plaque burden in 50 autopsy-confirmed Alzheimer’s disease patients. In the light of future biomarker applications like monitoring of disease progression and as allocortical neuropathological changes significantly contribute to clinical symptoms, the concept of in vivo surrogate biomarkers should be further explored.

Introduction

Recent studies suggested new potential applications for CSF biochemical markers (biomarkers) that reflect the neuropathology of Alzheimer’s disease (AD). Indeed, CSF β-amyloid peptide (Aβ1–42), total tau protein (T-tau) and hyperphosphorylated tau (P-tau) could find future applications in the prediction of the development of AD in mild cognitive impairment (MCI) patients, monitoring of AD disease progression and monitoring of the efficacy of disease-modifying treatment for AD (Tapiola et al., 2000; Riemenschneider et al., 2002; Andreasen et al., 2003; Stefanova et al., 2003; Wahlund and Blennow, 2003;
Zetterberg et al., 2003; Andreasen and Blennow, 2005; Hansson et al., 2006). These potential new applications urge the need for a better characterization of these biomarkers, preferably in autopsy-confirmed patients.

Although the €4 allele of APOE is a well-established risk factor for AD (Farrer et al., 1997), studies on possible associations between CSF biomarker levels and €4 produced conflicting results. In clinically diagnosed AD patients, several studies reported lower CSF $A_{\beta_{1-42}}$ levels in €4 carriers compared to non-carriers (Motter et al., 1995; Galasko et al., 1998; Hulstaert et al., 1999; Mehta et al., 2000; Riemenschneider et al., 2000; Andreasen et al., 2001; Csernansky et al., 2002; Prince et al., 2004; Sunderland et al., 2004) whereas others did not (Kunicki et al., 1998; Andreasen et al., 1999; Sjögren et al., 2000). Tapiola et al. (2000) found significantly lower CSF $A_{\beta_{1-42}}$ levels in clinically diagnosed AD patients carrying an €4 ($n=52$) compared to non-carriers ($n=28$), which was however not confirmed in a subset of autopsy-confirmed AD patients ($n=41$). In a post-mortem ventricular CSF study on autopsy-confirmed AD patients, no significant differences in $A_{\beta_{1-42}}$ levels between €4 carriers ($n=77$) and non-carriers ($n=48$) were found (Strozyk et al., 2003). Studying clinically diagnosed AD patients, no association of €4 and CSF T-tau levels could be demonstrated in the majority of studies (Lasser et al., 1998; Blomberg et al., 2001; Sunderland et al., 2004), whereas one study revealed significantly higher CSF T-tau levels in €4 carriers ($n=52$) compared to non-carriers ($n=28$), which was again not confirmed in a subset of autopsy-confirmed AD patients ($n=41$) (Tapiola et al., 2000). Although P-tau is a more specific biomarker for AD, studies on possible associations between €4 and CSF P-tau levels are sparse. In clinically diagnosed patients with AD ($n=67$), dementia with Lewy bodies (DLB) ($n=38$) and controls ($n=27$), no association between €4 and CSF P-tau$_{181P}$ was found (Vanderstichele et al., 2006). In 71 clinically diagnosed AD patients (Buerger et al., 2005) and in 26 definite AD patients (Buerger et al., 2006), no association between €4 and CSF P-tau$_{231P}$ could be demonstrated.

Few studies have investigated the relation between the CSF biomarkers $A_{\beta_{1-42}}$, T-tau and P-tau and neuropathological changes like plaque and tangle burden. In post-mortem collected ventricular CSF, lower levels of $A_{\beta_{1-42}}$ were associated with higher numbers of neuritic plaques in the neocortex of 155 definite AD patients (Strozyk et al., 2003). A positive correlation between CSF T-tau levels and neurofibrillary tangle (NFT) counts in the neocortex of AD patients was obtained (Tapiola et al., 1997). A recent study showed significant correlations of CSF P-tau$_{231P}$ levels with scores of NFT in several neocortical regions and with scores of neuritic plaques in frontal cortex of 26 definite AD patients (Buerger et al., 2006). Studies on possible associations with CSF P-tau$_{181P}$ are lacking so far.

In order to improve the characterization of the CSF biomarkers $A_{\beta_{1-42}}$, T-tau and P-tau$_{181P}$ and given the incomplete or conflicting data with regard to the possible associations with €4 and neuropathological variables, we set up a study in a well-characterized population with autopsy-confirmed AD, aiming to investigate possible associations of CSF $A_{\beta_{1-42}}$, T-tau and P-tau$_{181P}$ levels with €4 on the one hand and plaque and tangle burden on the other hand.

#### Materials and Methods

##### Study population

CSF samples from patients with autopsy-confirmed AD ($n=45$) or AD with cerebrovascular disease ($n=5$) and for whom APOE genotype was available, were retrieved from the Biobank, Institute Born-Bunge, Antwerp, Belgium. CSF samples were collected in clinical centers referring to the Biobank of the Institute Born-Bunge between April 1992 and July 2003. The study was approved by the local medical ethics committee.

##### Pathological criteria and neuropathological staging procedure

All pathological diagnoses were established by the same neuropathologist (JJM) who was blinded for the CSF results and for APOE genotyping. For AD patients, the neuropathological criteria of Braak and Braak (1991) and of Jellinger (1998) were used. Besides immunohistochemistry using AT8 against P-tau and 4G8 against Aβ+ amyloid, we systematically applied antibodies against ubiquitin to alleviate the need of unconventionally thick sections of 100 μm stained by Gallyas’ silver technique as proposed in the revised staging procedure of Braak et al. (2006). AD with cerebrovascular disease was diagnosed according to Markesbery (1998).

Tangle burden was assessed by means of Braak’s NFT stages (I–VI) whereas plaque burden was assessed by means of Braak’s senile plaque (SP) stages (A–C). Based on neuropathological criteria of Braak and Braak (1991), complemented by Jellinger (1998) and Braak et al. (2006), distributions and semi-quantitatively rated densities of NFT and SP were taken into account to assign NFT and SP stages. Indeed, the revised staging procedure of Braak et al. (2006) allowed the use of paraffin sections of conventional thickness. Following immunostaining and using a standard grid of $680 \times 980 \mu m$ at four random fields in the required neocortical and allocortical regions, this procedure allowed us to semi-quantitatively score SP and NFT densities.

##### APOE genotyping

Genomic DNA was extracted from total blood using standard methods and the APOE genotype was determined as described earlier (Slooter et al., 1998). Allele frequencies were assessed by counting alleles and calculating proportions.

##### CSF sampling and storage

CSF was obtained in referring clinical centres during clinical work-up of the patient by lumbar puncture at the L3/L4 or L4/L5 interspace. A minimum sample volume of 1 ml was collected and stored at −20°C or lower until analysis.
CSF analysis

CSF analysis was performed at the Innogenetics R&D facilities (Ghent, Belgium) following relabelling of the CSF vials. The laboratory technician was blinded for results of APOE genotyping and for the expected test outcome in terms of clinical and definitive pathological diagnoses when performing and interpreting the tests.

CSF levels of $A\beta_{1-42}$, T-tau, and P-tau$\text{P}_{181}$ were determined with commercially available single-parameter ELISA kits (respectively INNOTEST® $\beta$-AMYLOID$\text{P}_{1-42}$, INNOTEST® hTAUAg, INNOTEST® PHOSPHO-THAU$\text{P}_{181}$, Innogenetics, Ghent, Belgium). With each assay, the clinical samples, together with a blank (sample diluent), the (prepared) calibrator solutions and the appropriate controls, were tested strictly following the instructions provided in the kit inserts. All samples were run in duplicate. If the intra-assay coefficient of variance was >30% (calculated as range/100/average), or if concentrations obtained were out-of-range (OD values not between mean OD values of highest and lowest calibration concentration), samples were retested (by extension of the calibrator concentration range for some samples). Dilution of samples with measured concentrations above the highest calibration concentration for possible reanalysis was not performed, as this is not recommended in the manufacturer’s instructions. The concentration ranges of the test kits are described in the package inserts ($A\beta_{1-42}$: 15.6–500 pg/ml, T-tau: 75–1200 pg/ml, $A\beta_{1-42}$: 125–2000 pg/ml).

Statistical analyses

Data were compared using student’s $t$-test [or Mann–Whitney Rank Sum Test (RST) when lacking normal distribution] or Chi-square statistics. Spearman’s Rank Order was used for correlation calculation. A hypothesis test was considered significant if its associated $P$-value was $<$0.05. Analyses were performed using SigmaStat software and SPSS 13.0 (SPSS Science, Erkrath, Germany).

Results

Description of the study population

The study population consisted of 24 males and 26 females. Demographic and clinical data as well as APOE genotype and allele frequencies and neuropathological data are summarized in Table 1.

The majority (36/49, 73%) of CSF samples were taken within 1 year preceding death. Date of CSF sampling was unknown for one patient. A highly significant and positive correlation between plaque and tangle burden was found ($r = 0.610, P < 0.001$). Three AD patients were identified as members of known AD families. Two patients carried the PSEN1 Ile143Thr mutation (Cruts et al., 1995), whereas one patient carried the PSEN1 Leu282Val mutation (Dermaut et al., 2001).

### Table 1 Demographic, clinical, biomarker, genetic and neuropathological data

<table>
<thead>
<tr>
<th>Demographic, clinical and biomarker data</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Age at CSF sampling (years)</td>
<td>49</td>
<td>75.9</td>
<td>12.1</td>
<td>34–94</td>
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<td>Age at autopsy (years)</td>
<td>50</td>
<td>76.2</td>
<td>12.4</td>
<td>39–94</td>
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<td>MMSE at CSF sampling (/30)</td>
<td>45</td>
<td>10.5</td>
<td>6.2</td>
<td>0–25</td>
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<tr>
<td>Interval CSF sampling–autopsy (months)</td>
<td>49</td>
<td>12.6</td>
<td>19.5</td>
<td>0.25–65</td>
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<td>CSF $A\beta_{1-42}$ levels (pg/ml)</td>
<td>50</td>
<td>362.7</td>
<td>196.2</td>
<td>1970–718.0</td>
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<tr>
<td>CSF P-tau$\text{P}_{181}$ levels (pg/ml)</td>
<td>50</td>
<td>85.5</td>
<td>53.6</td>
<td>21.4–327.3</td>
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<tr>
<td>CSF tau levels (pg/ml)</td>
<td>50</td>
<td>686.0</td>
<td>360.9</td>
<td>92.0–1200.0</td>
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<td>CSF tau levels (pg/ml) (out-of-range data set equal to highest calibration concentration)</td>
<td>41</td>
<td>573.2</td>
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<td>92.0–1194.0</td>
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**Genetic and neuropathological data**

<table>
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<th>Relative no (%)</th>
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<tr>
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<td>6</td>
</tr>
<tr>
<td>$e2/e4$</td>
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<td>2</td>
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<tr>
<td>$e3/e3$</td>
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<tr>
<td>$e3/e4$</td>
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<table>
<thead>
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<tr>
<td>$e3$</td>
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<tr>
<td>$e4$</td>
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<table>
<thead>
<tr>
<th>Braak’s NFT stages (I–VI)</th>
<th>n</th>
<th>Absolute no</th>
<th>Relative no (%)</th>
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<tbody>
<tr>
<td>I</td>
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<td>2</td>
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</tr>
<tr>
<td>II</td>
<td>8</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>IV</td>
<td>14</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>VI</td>
<td>21</td>
<td>42</td>
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</table>

<table>
<thead>
<tr>
<th>Braak’s SP stages (A–C)</th>
<th>n</th>
<th>Absolute no</th>
<th>Relative no (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>29</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>
**CSF sample characteristics, CSF analyses and CSF biomarker levels**

Five CSF samples underwent two freeze-thaw cycles, whereas all other samples had never been thawed before analysis. The composition of the storage tube was polystyrene for one sample, unknown for three samples and polypropylene for all other samples. Nine samples showed too high (out-of-range) results for T-tau after retesting. Therefore, all data analyses concerning CSF biomarker levels were performed twice: (1) with out-of-range data set equal to the highest T-tau calibration concentration (1200 pg/ml) and (2) without out-of-range concentrations that were considered missing data. As the CSF biomarker levels were not affected by age, gender or MMSE score at CSF sampling (data not shown), raw biomarker concentrations were used for data analyses in the present study. CSF biomarker levels are summarized in Table 1.

**No associations of ε4 with clinical data and biomarker levels**

No significant differences were found when age at CSF sampling, age at autopsy, MMSE at CSF sampling and CSF biomarker levels were compared between ε4 carriers and non-carriers (Table 2).

No significant correlations between number of ε4 alleles (0, 1 or 2) and age at CSF sampling (\(r = 0.050, P = 0.730\)), age at autopsy (\(r = 0.016, P = 0.910\)), MMSE at CSF sampling (\(r = -0.067, P = 0.662\)), CSF levels of Aβ\(_{1–42}\) (\(r = -0.057, P = 0.695\)), T-tau (\(r = 0.104, P = 0.472\)) and P-tau\(_{181P}\) (\(r = 0.062, P = 0.668\)) were found. Repeating correlation calculation excluding out-of-range CSF T-tau levels did not reveal significant associations either (\(r = -0.006, P = 0.972\)).

**No associations of plaque and tangle burden with CSF biomarker levels**

Braak’s SP (Aβ\(_{1–42}\): \(r = -0.155, P = 0.280\); T-tau: \(r = -0.044, P = 0.763\); P-tau\(_{181P}\): \(r = -0.010, P = 0.947\)) and NFT (Aβ\(_{1–42}\): \(r = -0.145, P = 0.315\); T-tau: \(r = 0.117, P = 0.415\); P-tau\(_{181P}\): \(r = 0.150, P = 0.296\)) stages were not significantly correlated with CSF biomarker levels. Repeating correlation calculation excluding out-of-range CSF T-tau levels did not reveal significant associations with plaque (\(r = -0.015, P = 0.923\)) and tangle burden (\(r = 0.120, P = 0.453\)) either.

Comparing a subgroup of patients categorized into Braak’s NFT stages I to IV (\(n = 26\)) with a subgroup of patients categorized into stages V and VI (\(n = 24\)), no significant differences in CSF levels of Aβ\(_{1–42}\), T-tau (with and without out-of-range concentrations) and P-tau\(_{181P}\) were found (data not shown). The same held true for the comparison of a subgroup categorized into Braak’s SP stages A and B (\(n = 30\)) compared to patients belonging to stage C (\(n = 20\)).

**Discussion**

**Study design, CSF biomarker levels**

The present data set is unique given the number of well-characterized and pathologically confirmed AD patients with autemortem CSF sampling and APOE genotyping which gave us the chance to study possible associations with a complete panel of the three most frequently used CSF biomarkers in one and the same population.

The number of samples with out-of-range concentrations for T-tau was rather high in this study. Although there is no straightforward explanation, it can be speculated that the observation of too high CSF T-tau results can by itself be indicative of an underlying AD.

**No associations of ε4 with clinical data and biomarker levels**

Studies investigating possible associations of ε4 (carrier versus non-carrier status and the number of alleles) with CSF levels of Aβ\(_{1–42}\) produced conflicting results (Motter et al., 1995; Galasko et al., 1998; Kunicki et al., 1998; Andreasen et al., 1999; Hulstaert et al., 1999; Andreasen et al., 1999).

**Table 2** Comparison of demographic, clinical and biochemical data between ε4 carriers and non-carriers

<table>
<thead>
<tr>
<th></th>
<th>ε4 carriers (n = 21)</th>
<th>ε4 non-carriers (n = 29)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at CSF sampling (years)</td>
<td>76.9 ± 11.1*</td>
<td>74.6 ± 13.6</td>
<td>RST: T = 496.5; P = 0.572</td>
</tr>
<tr>
<td>Age at autopsy (years)</td>
<td>76.5 ± 12.2</td>
<td>75.9 ± 13.0</td>
<td>RST: T = 520.0; P = 0.768</td>
</tr>
<tr>
<td>MMSE at CSF sampling (30)</td>
<td>101.0 ± 6.0</td>
<td>111.1 ± 6.7</td>
<td>t-test: t = 0.524; df = 43; P = 0.603</td>
</tr>
<tr>
<td>CSF Aβ(_{1–42}) levels (pg/ml)</td>
<td>368.1 ± 11.0</td>
<td>355.1 ± 101.3</td>
<td>t-test: t = -0.424; df = 48; P = 0.674</td>
</tr>
<tr>
<td>CSF P-tau(_{181P}) levels (pg/ml)</td>
<td>874.8 ± 44.8</td>
<td>82.8 ± 65.0</td>
<td>RST: T = 488.0; P = 0.356</td>
</tr>
<tr>
<td>CSF tau levels (pg/ml) (out-of-range data set equal to highest calibration concentration)</td>
<td>741.8 ± 364.3</td>
<td>6090 ± 350.0</td>
<td>t-test: t = -1.293; df = 48; P = 0.202</td>
</tr>
<tr>
<td>CSF tau levels (pg/ml) (out-of-range data considered as missing data)</td>
<td>596.0 ± 291.1</td>
<td>546.8 ± 305.3</td>
<td>t-test: t = -0.529; df = 39; P = 0.601</td>
</tr>
</tbody>
</table>

*Note: Data are given as mean ± SD. A student’s t-test or—it lacking normal distribution—a Mann–Whitney Rank Sum Test (RST) was used for comparing data between ε4 carriers and non-carriers.

*Age at CSF sampling was unknown for one patient (autopsied at age 44 years) and was considered as missing data for statistical analysis.
Mehta et al., 2000; Riemenschneider et al., 2000; Sjögren et al., 2000; Tapiola et al., 2000; Andreasen et al., 2001; Csernansky et al., 2002; Prince et al., 2004; Sunderland et al., 2004). These might amongst others be attributable to aetiological heterogeneity of study populations as all but two studies (Tapiola et al., 2000; Strozyk et al., 2003) were performed in clinically diagnosed populations. In our population of 50 patients with definite AD, we could not demonstrate a significant association between 

\[ \beta_4 \] 

and CSF A\(_\beta_4\) levels either, which is in accordance with both formerly published studies in autopsy-confirmed AD patients (Tapiola et al., 2000; Strozyk et al., 2003).

We did not find significant associations between 

\[ \beta_4 \] 

and CSF levels of T-tau, thus confirming formerly published negative studies in clinically diagnosed AD patients (Lasser et al., 1998; Blomberg et al., 2001; Sunderland et al., 2004) and one study with a subset of 41 autopsy-confirmed AD patients (Tapiola et al., 2000). The present study demonstrated the lack of an association between 

\[ \beta_4 \] 

and CSF levels of P-tau181P, which is in accordance with a study on P-tau181P in 67 clinically diagnosed AD patients (Vanderstichele et al., 2006) and a study on P-tau231P in 71 clinically diagnosed (Buerger et al., 2005) and 26 definite AD patients (Buerger et al., 2006).

In conclusion, our study is not indicative for associations between CSF levels of A\(_\beta_1-42\), T-tau and P-tau181P and 

\[ \beta_4 \] 

e in a population of 50 definite AD patients. As an association study in 50 subjects might be underpowered in case of rather weak associations which might apply to APOE these negative findings await confirmation in an extended population of autopsy-confirmed AD cases.

**No associations of plaque and tangle burden with CSF biomarker levels**

Possible associations between the CSF biomarkers A\(_\beta_1-42\), T-tau and P-tau181P and plaque and tangle burden were investigated. Although these CSF biomarkers reflect AD’s neuropathology and despite the fact that the majority of CSF samples were taken within 1 year preceding death, no significant associations between the CSF levels of these biomarkers and Braak’s SP and NFT stages were found in a population of 50 definite AD patients. In order to rule out that cases with long intervals between CSF sampling and autopsy have biased the results, the lack of significant correlations between CSF biomarker levels and Braak’s SP and NFT stages was confirmed in a subset of patients (n = 36) with short intervals between CSF sampling and autopsy (≤12 months) (data not shown).

These findings are in contrast with the three formerly published studies dealing with associations between CSF biomarker levels and amyloid- or tau-neuropathology (Tapiola et al., 2000; Strozyk et al., 2003; Buerger et al., 2006). Indeed, Strozyk et al. (2003) and Tapiola et al. (1997) respectively described significant associations between CSF levels of A\(_\beta_1-42\) and T-tau with SP and NFT counts in selected brain regions. Although publications on possible associations with CSF P-tau181P are lacking so far, a recent study showed significant correlations of CSF P-tau231P levels with scores of NFT in several neocortical regions but not in hippocampus and with scores of neuritic plaques in frontal cortex but not in temporal, parietal and hippocampal cortical areas of 26 definite AD patients (Buerger et al., 2006).

How can these seemingly conflicting data be explained? First, differences in patient characteristics might have contributed to the differences between the present and formerly published studies. Indeed, Tapiola et al. (1997) and Buerger et al. (2006) only included patients with clinically severe AD whereas our study population also contained patients at the moderate dementia stages. As there was a range of up to 9 years between clinical assessment and autopsy in the study of Strozyk et al. (2003), it is hard to judge the clinical dementia stage at death of the patient population they included. Methodological differences were obvious as well. Indeed, Strozyk et al. (2003) used post-mortem ventricular CSF meanwhile revealing negative correlations between CSF A\(_\beta_1-42\) levels and time intervals between death and autopsy. Moreover, as the study of Buerger et al. (2006) only included 26 definite AD patients, small sample size might have limited statistical validity so that one cannot rule out that positive findings are due to some outliers.

Another methodological difference concerns the procedures that were used to assess tangle burden. By applying immunohistochemistry (present study and study of Buerger et al. (2006)), only P-tau in NFT is considered whereas the determination of P-tau in brain homogenates (study of Buerger et al. (2006)) also takes other P-tau sources (like P-tau in axons) into account. Indeed, it cannot be ruled out that P-tau181P and P-tau231P are distributed differently in neurons and NFT-related processes. As AT8 immunostaining has been used in both studies to determine densities of NFT, discrepancies in correlations between CSF biomarker levels and tangle burden cannot be explained by the use of different immunostaining techniques. However, the major difference that might have contributed to the seemingly discrepant study results is the neuropathological assessment procedure that was applied to determine tangle burden. While Buerger et al. (2006) determined tangle burden in selected neocortical brain regions, we used Braak’s NFT stages, taking into account extent and localization in neocortical and allocortical areas. The fact that these different methodologies resulted in different findings is intriguing and might be of significant (pathophysiological) importance with regard to the concept of *in vivo* surrogate biomarkers.

Given the fact that CSF turnover is high, changes in CSF biomarker levels most probably reflect dynamic changes in the brain. Indeed, results from a recent study indicated that CSF levels of brain-derived proteins correlated with the degree of neuronal damage. However, this association is
modified by the localization of the brain pathology (Boesenberg-Grosse et al., 2006). Although we did not find significant correlations between CSF biomarkers levels and Braak’s NFT stages in a subpopulation of patients belonging to stages I and II \( (n = 9) \) (data not shown), it can be hypothesized that correlations between CSF biomarker levels and tangle burden are mainly the reflection of neurodegeneration in the temporal lobe and that once the temporal accumulation of SP and NFT has reached a plateau and subsequently spreads to other brain regions, no correlation is revealed anymore. Calculating correlations between CSF P-tau\(_{231}\)P levels and neurofibrillary pathology using Braak NFT stages of the dataset of Buerger et al. (2006) (cfr. table 1 of Buerger et al., 2006), no significant associations can be found either (Fig. 1B), especially when stage I and II cases are left out (3 of 26). This is completely in line with our findings (Fig. 1A), meanwhile indicating that future studies aiming to correlate CSF biomarkers with neuropathological variables should perform quantifications of neuropathological changes in selected neocortical brain regions. The more so as levels of A\( \beta \), T-tau and P-tau in the medial temporal cortex of AD patients significantly correlated with Braak’s NFT stages (Zhou et al., 2006), our findings might have identified possible limits of the application of CSF A\( \beta \), T-tau and P-tau levels as in vivo surrogate biomarkers for AD as was recently reviewed by Nichols et al. (2006) with regard to A\( \beta \). In the light of future biomarker applications like monitoring of disease progression and as allocortical neuropathological changes significantly contribute to the clinical symptoms AD patients display (Braak et al., 1999), the concept of in vivo surrogate biomarkers should be further explored in order to understand the relationship between circulating biomarkers and pathological mechanisms in the brain.

Conclusions

In 50 autopsy-confirmed AD patients, CSF levels of A\( \beta \)\(_{1–42}\), T-tau and P-tau\(_{181}\)P were not associated with APOE e4 and were not correlated with density and spread of NFT and SP as assessed by Braak’s NFT and SP stages.

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Competing Interests:

E.V. is an employee at Innogenetics NV.

References


