

# No association of CSF biomarkers with APOE $\epsilon$ 4, plaque and tangle burden in definite Alzheimer's disease

Sebastiaan Engelborghs,<sup>1,2,6,7</sup> Kristel Slegers,<sup>3,6,8</sup> Patrick Cras,<sup>4,6,9</sup> Nathalie Brouwers,<sup>3,6,8</sup> Sally Serneels,<sup>3,6,8</sup> Evelyn De Leenheir,<sup>5</sup> Jean-Jacques Martin,<sup>5</sup> Eugeen Vanmechelen,<sup>10</sup> Christine Van Broeckhoven<sup>3,6,8</sup> and Peter Paul De Deyn<sup>1,2,5,6</sup>

<sup>1</sup>Department of Neurology and Memory Clinic, Middelheim General Hospital (ZNA), <sup>2</sup>Reference Centre for Biological Markers of Memory Disorders, Laboratory of Neurochemistry and Behavior, <sup>3</sup>Laboratory of Neurogenetics, <sup>4</sup>Laboratory of Neurobiology and <sup>5</sup>Biobank, Institute Born-Bunge, <sup>6</sup>University of Antwerp, Antwerpen, Belgium, <sup>7</sup>Department of Nursing Sciences, Faculty of Medicine, University of Antwerp, Antwerpen, Belgium, <sup>8</sup>Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerpen, Belgium, <sup>9</sup>Department of Neurology, University Hospital of Antwerp, Antwerpen, Belgium and <sup>10</sup>Innogenetics NV, Gent, Belgium

Correspondence to: Prof. Dr P. P. De Deyn, Laboratory of Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Universiteitsplein I, BE-2610 Antwerp, Belgium  
E-mail: peter.dedeyn@ua.ac.be.

**The CSF biomarkers  $\beta$ -amyloid peptide ( $A\beta_{1-42}$ ), total tau protein (T-tau) and tau phosphorylated at threonine 181 (P-tau<sub>181P</sub>) were determined in autopsy-confirmed Alzheimer's disease patients in order to study possible associations with the  $\epsilon$ 4 allele of APOE and density and spread of plaques (SP) and tangles (NFT).**

**CSF levels of  $A\beta_{1-42}$ , T-tau and P-tau<sub>181P</sub> 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  $\epsilon$ 4 carriers ( $n=21$ ) and non-carriers ( $n=29$ ) ( $P>0.05$  for all comparisons). No significant correlations between the number of  $\epsilon$ 4 alleles (0, 1 or 2) and CSF levels of  $A\beta_{1-42}$  (Spearman Rank Order:  $r=-0.057$ ,  $P=0.695$ ), T-tau ( $r=0.104$ ,  $P=0.472$ ) and P-tau<sub>181P</sub> ( $r=0.062$ ,  $P=0.668$ ) were found. Braak's SP ( $A\beta_{1-42}$ :  $r=-0.155$ ,  $P=0.280$ ; T-tau:  $r=-0.044$ ,  $P=0.763$ ; P-tau<sub>181P</sub>:  $r=-0.010$ ,  $P=0.947$ ) and NFT ( $A\beta_{1-42}$ :  $r=-0.145$ ,  $P=0.315$ ; T-tau:  $r=0.117$ ,  $P=0.415$ ; P-tau<sub>181P</sub>:  $r=0.150$ ,  $P=0.296$ ) stages were not significantly correlated with CSF biomarker levels.**

**In conclusion, CSF levels of  $A\beta_{1-42}$ , T-tau and P-tau<sub>181P</sub> were not associated with  $\epsilon$ 4, 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.**

**Keywords:** dementia; Alzheimer's disease; biomarkers; CSF; APOE

**Abbreviations:**  $A\beta_{1-42}$  =  $\beta$ -amyloid peptide; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; MCI = mild cognitive impairment; NFT = neurofibrillary tangle; P-tau = hyperphosphorylated tau; SP = senile plaque; T-tau = total tau protein

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## Introduction

Recent studies suggested new potential applications for CSF biochemical markers (biomarkers) that reflect the neuropathology of Alzheimer's disease (AD). Indeed, CSF  $\beta$ -amyloid peptide ( $A\beta_{1-42}$ ), 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  $\epsilon 4$  allele of APOE is a well-established risk factor for AD (Farrer *et al.*, 1997), studies on possible associations between CSF biomarker levels and  $\epsilon 4$  produced conflicting results. In clinically diagnosed AD patients, several studies reported lower CSF  $A\beta_{1-42}$  levels in  $\epsilon 4$  carriers compared to non-carriers (Mottter *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  $\epsilon 4$  ( $n=52$ ) 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  $\epsilon 4$  carriers ( $n=77$ ) and non-carriers ( $n=48$ ) were found (Strozyk *et al.*, 2003). Studying clinically diagnosed AD patients, no association of  $\epsilon 4$  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  $\epsilon 4$  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  $\epsilon 4$  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  $\epsilon 4$  and CSF P-tau<sub>181P</sub> 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  $\epsilon 4$  and CSF P-tau<sub>231P</sub> 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<sub>231P</sub> 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<sub>181P</sub> are lacking so far.

In order to improve the characterization of the CSF biomarkers  $A\beta_{1-42}$ , T-tau and P-tau<sub>181P</sub> and given the incomplete or conflicting data with regard to the possible associations with  $\epsilon 4$  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<sub>181P</sub> levels with  $\epsilon 4$  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  $A\beta$  + amyloid, we systematically applied antibodies against ubiquitin to alleviate the need of unconventionally thick sections of 100  $\mu\text{m}$  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  $\mu\text{m}$  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  $-20^{\circ}\text{C}$  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<sub>181P</sub> were determined with commercially available single-parameter ELISA kits (respectively INNOTEST<sup>®</sup>  $\beta$ -AMYLOID<sub>(1-42)</sub>, INNOTEST<sup>®</sup> hTAUAg, INNOTEST<sup>®</sup> PHOSPHO-TAU<sub>(181P)</sub>, 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  $\times$  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 (P-tau<sub>181P</sub>: 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

Demographic, clinical and biomarker data	<i>n</i>	Mean	SD	Range
Age at CSF sampling (years)	49	75.9	12.1	34–94
Age at autopsy (years)	50	76.2	12.4	39–94
MMSE at CSF sampling (/30)	45	10.5	6.2	0–25
Interval CSF sampling—autopsy (months)	49	12.62	19.54	0.25–65
CSF A $\beta_{1-42}$ levels (pg/ml)	50	362.7	106.2	197.0–718.0
CSF P-tau <sub>181P</sub> levels (pg/ml)	50	85.5	53.6	21.4–327.3
CSF tau levels (pg/ml) (out-of-range data set equal to highest calibration concentration)	50	686.0	360.9	92.0–1200.0
CSF tau levels (pg/ml) (out-of-range data considered as missing data)	41	573.2	295.0	92.0–1194.0
<b>Genetic and neuropathological data</b>		<b>Absolute no</b>	<b>Relative no (%)</b>	
APOE genotype	$\epsilon 2/\epsilon 2$	0	0	
	$\epsilon 2/\epsilon 3$	3	6	
	$\epsilon 2/\epsilon 4$	1	2	
	$\epsilon 3/\epsilon 3$	18	36	
	$\epsilon 3/\epsilon 4$	23	46	
	$\epsilon 4/\epsilon 4$	5	10	
APOE allele	$\epsilon 2$	4	4	
	$\epsilon 3$	62	62	
	$\epsilon 4$	34	34	
Braak's NFT stages (I–VI)	I	1	2	
	II	8	16	
	III	3	6	
	IV	14	28	
	V	3	6	
	VI	21	42	
Braak's SP stages (A–C)	A	1	2	
	B	29	58	
	C	20	40	

### 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 $\epsilon 4$ 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  $\epsilon 4$  carriers and non-carriers (Table 2).

No significant correlations between number of  $\epsilon 4$  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  $A\beta_{1-42}$  ( $r = -0.057$ ,  $P = 0.695$ ), T-tau ( $r = 0.104$ ,  $P = 0.472$ ) and P-tau<sub>181P</sub> ( $r = 0.062$ ,  $P = 0.668$ ) 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 ( $A\beta_{1-42}$ :  $r = -0.155$ ,  $P = 0.280$ ; T-tau:  $r = -0.044$ ,  $P = 0.763$ ; P-tau<sub>181P</sub>:  $r = -0.010$ ,  $P = 0.947$ )

and NFT ( $A\beta_{1-42}$ :  $r = -0.145$ ,  $P = 0.315$ ; T-tau:  $r = 0.117$ ,  $P = 0.415$ ; P-tau<sub>181P</sub>:  $r = 0.150$ ,  $P = 0.296$ ) 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 or VI ( $n = 24$ ), no significant differences in CSF levels of  $A\beta_{1-42}$ , T-tau (with and without out-of-range concentrations) and P-tau<sub>181P</sub> 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 antemortem 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 $\epsilon 4$ with clinical data and biomarker levels

Studies investigating possible associations of  $\epsilon 4$  (carrier versus non-carrier status and the number of alleles) with CSF levels of  $A\beta_{1-42}$  produced conflicting results (Motter *et al.*, 1995; Galasko *et al.*, 1998; Kunicki *et al.*, 1998; Andreasen *et al.*, 1999; Hulstaert *et al.*, 1999;

**Table 2** Comparison of demographic, clinical and biochemical data between  $\epsilon 4$  carriers and non-carriers

	$\epsilon 4$ carriers ( $n = 21$ )	$\epsilon 4$ non-carriers ( $n = 29$ )	Statistical analysis
Age at CSF sampling (years)	76.9 $\pm$ 11.1 <sup>a</sup>	74.6 $\pm$ 13.6	RST: $T = 496.5$ ; $P = 0.572$
Age at autopsy (years)	76.5 $\pm$ 12.2	75.9 $\pm$ 13.0	RST: $T = 520.0$ ; $P = 0.768$
MMSE at CSF sampling (/30)	10.1 $\pm$ 6.0	11.1 $\pm$ 6.7	t-test: $t = 0.524$ ; $df = 43$ ; $P = 0.603$
CSF $A\beta_{1-42}$ levels (pg/ml)	368.1 $\pm$ 111.0	355.1 $\pm$ 101.3	t-test: $t = -0.424$ ; $df = 48$ ; $P = 0.674$
CSF P-tau <sub>181P</sub> levels (pg/ml)	87.4 $\pm$ 44.8	82.8 $\pm$ 65.0	RST: $T = 488.0$ ; $P = 0.356$
CSF tau levels (pg/ml) (out-of-range data set equal to highest calibration concentration)	741.8 $\pm$ 364.3	609.0 $\pm$ 350.0	t-test: $t = -1.293$ ; $df = 48$ ; $P = 0.202$
CSF tau levels (pg/ml) (out-of-range data considered as missing data)	596.0 $\pm$ 291.1	546.8 $\pm$ 305.3	t-test: $t = -0.528$ ; $df = 39$ ; $P = 0.601$

Note: Data are given as mean  $\pm$  SD. A student's t-test or—when lacking normal distribution—a Mann–Whitney Rank Sum Test (RST) was used for comparing data between  $\epsilon 4$  carriers and non-carriers.

<sup>a</sup>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  $\epsilon 4$  and CSF  $A\beta_{1-42}$  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  $\epsilon 4$  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  $\epsilon 4$  and CSF levels of P-tau<sub>181P</sub>, which is in accordance with a study on P-tau<sub>181P</sub> in 67 clinically diagnosed AD patients (Vanderstichele *et al.*, 2006) and a study on P-tau<sub>231P</sub> 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-tau<sub>181P</sub> and  $\epsilon 4$  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-tau<sub>181P</sub> 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 ( $\leq 12$  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-tau<sub>181P</sub> are lacking so far, a recent study showed significant correlations of CSF P-tau<sub>231P</sub> 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-tau<sub>181P</sub> and P-tau<sub>231P</sub> 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<sub>231P</sub> 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 $\beta$ <sub>1-42</sub>, T-tau and P-tau<sub>181P</sub> were not associated with APOE  $\epsilon$ 4 and were not correlated with density and spread of NFT and SP as assessed by Braak's NFT and SP stages.

## Acknowledgements

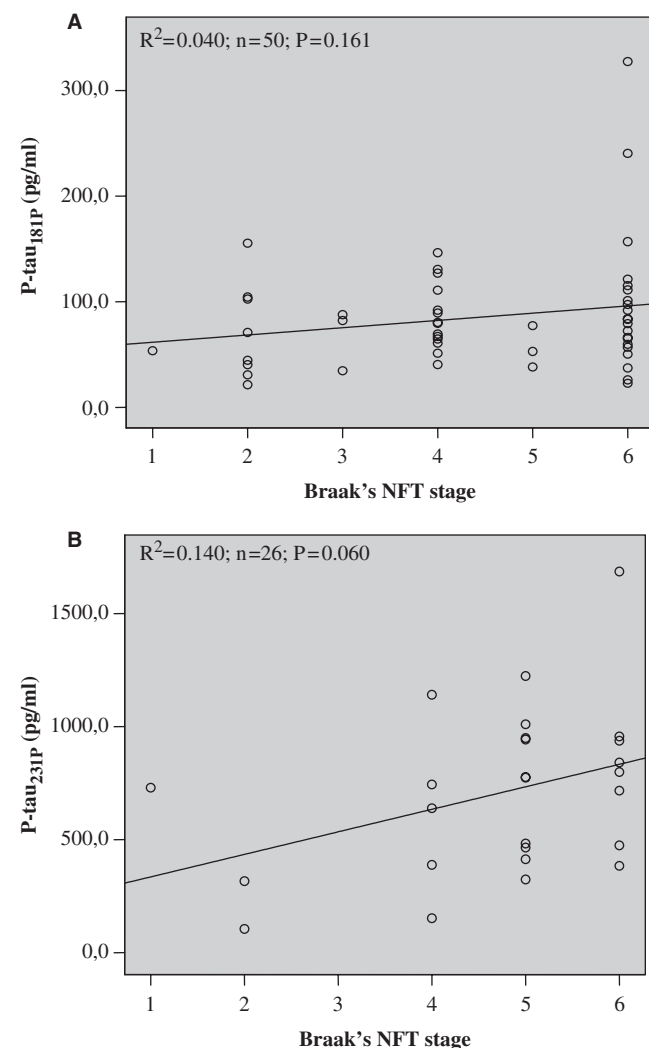
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## Competing interests:

E.V. is an employee at Innogenetics NV.

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**Fig. 1** (A) Scatter plot for CSF P-tau<sub>181P</sub> levels as a function of Braak's NFT stages. (B) Scatter plot for CSF P-tau<sub>231P</sub> levels as a function of Braak's NFT stages (data from Buerger *et al.*, 2006).

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