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Evaluation of a novel immunoassay to detect p-Tau Thr127 in the CSF to distinguish Alzheimer disease from other dementias

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DISCLOSURE

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Objective

To investigate whether p-tau T217 assay in cerebrospinal fluid (CSF) can distinguish Alzheimer's disease from other dementias and healthy controls.

Methods

We developed and validated a novel Simoa immunoassay to detect p-tau T217 in CSF. There was a total of 190 participants from three cohorts with AD (n = 77) and other neurodegenerative diseases (n = 69) as well as healthy subjects (n = 44).

Results

The p-tau T217 assay (cut-off 242 pg/ml) identified AD subjects with accuracy of 90%, with 78% positive predictive value (PPV), 97% negative predictive value (NPV), 93% sensitivity, 88% specificity compared favorably with p-tau T181 ELISA (52 pg/ml) showing 78% accuracy, 58% PPV, 98% NPV, 71% specificity, 97% sensitivity. The assay distinguished AD patients from age-matched healthy subjects (cut-off 163 pg/ml, sensitivity 98%, specificity 93%) similarly to p-tau T181 ELISA (cut-off 60 pg/ml, 96% sensitivity and 86% specificity). In AD patients, we found a strong correlation between p-tau T217-tau and p-tau T181, t-tau and A β 40 but not with A β 42.

Conclusions

This study demonstrates that p-tau T217 displayed better diagnostic accuracy than p-tau T181. The data suggests that the new p-tau T217 assay has a potential as an AD diagnostic test in the clinical evaluation.

Classification of Evidence:

This study provides Class III evidence that a CSF immunoassay for p-tau T217 distinguishes AD from other dementias and healthy controls.

Glossary: **AD** = Alzheimer's disease; **A β** = amyloid β ; **t-tau** = total-tau; **NfL** = neurofilament light chain; **NSE** = neuron specific enolase; **YKL-40** = chitinase-like glycoprotein; **MCP-1** = monocyte chemoattractant protein-1; **PHF-tau** = sarkosyl-insoluble tau; **LLOQ** = lower limit of quantification; **nvPPA** = non-fluent variant of primary progressive aphasia; **svPPA** = semantic variant of PPA; **bvFTD** = behavioural variant of frontotemporal dementia; **PSP** = primary progressive palsy; **CBD** = corticobasal degeneration; **MMSE** = Mini-Mental State Examination; **AUC** = area under the curve; **CI** = confidence interval

INTRODUCTION

In Alzheimer's disease (AD), there is a need for biomarkers that reflect the key pathophysiology of the disease: neurodegeneration; amyloid β ($A\beta$) and tau protein pathology¹. Over the last two decades, significant efforts have been made to identify *in vivo* brain indicators and fluid-based biomarkers for preclinical and clinical AD²⁻⁴.

In 2018, the National Institute on Aging and Alzheimer's Association Research Framework shifted the definition of AD in living people from a syndromal to a biological construct⁵. The new research framework defines AD by using a variety of biomarkers, which are grouped into those of amyloid β deposition, pathologic tau protein, and neurodegeneration (A/T/N)⁵. Recently, a new biomarker for AD has been reported – tau phosphorylated at Thr217 (p-tau T217). It has been shown that p-tau T217 species (quantified as pT217/ T217 ratio) highly correlate with amyloid lesions in the brain, cognitive decline and tau PET imaging in AD⁶⁻⁸. In physiological conditions, p-tau T217 species display rapid turnover in the extracellular space⁸.

We developed a novel immunoassay to detect p-tau T217 in the CSF, and subsequently undertook this study to 1) evaluate the sensitivity and specificity of the p-tau T217 assay using AD and non-AD CSF samples from three international cohorts of patients; 2) compare its sensitivity and specificity with standard CSF measures (particularly with t-tau, p-tau T181, $A\beta$ 40 and $A\beta$ 42); and 3) assess the relationship between the levels of p-tau T217 with above-mentioned standard CSF biomarkers.

MATERIAL AND METHODS

ASSAY DEVELOPMENT AND VALIDATION

Preparation of hybridoma cell lines expressing DC2E7 and DC2E2 antibodies and their purification

We prepared DC2E7 and DC2E2 hybridoma cell lines as described previously⁹. To generate the DC2E7 and DC2E2 antibodies, we immunized Balb/c mice with either sarkosyl-insoluble tau (PHF-tau) isolated from AD brain (frontal cortex, Braak stage VI, Netherlands brain bank), or with recombinant human tau protein (aa 1–242 of the longest tau isoform).

Antibodies were purified using protein-G affinity chromatography by Äkta Avant Purifier (both GE Healthcare).

Analyzing of both antibodies showed that DC2E7 recognizes phosphorylated tau protein, while DC2E2 is a pan-tau antibody recognizing a proline-rich domain on tau protein. To define the exact phosphoepitope for DC2E7, we generated mutated forms of tau 2N4R with single point mutations in which serine and threonine residues were replaced by alanine. The immunoblotting analysis showed that antibody DC2E7 recognized all phosphorylated tau proteins carrying the point mutations except for Thr217Ala. This suggests that phospho-threonine at position 217 creates a key part of the epitope recognized by antibody DC2E7.

Data available from Dryad (Suppl. Fig.1): <https://doi.org/10.5061/dryad.tdz08kpwz>.

p-tau T217 Simoa assay

We set up the p-tau T217 assay in the highly sensitive format of a Single molecule array (Simoa) digital ELISA, using an HD-1 Analyzer (Quanterix, Billerica, USA). Reagents for the assay were prepared according to the Quanterix Homebrew Assay Development Guide with the following details: we used DC2E7 antibody as a capture antibody and DC2E2 antibody as

a detector antibody; the capture antibody DC2E7 was coupled to magnetic beads (Quanterix) at a concentration of 0.5 mg/ml according to the Simoa alternate bead conjugation protocol (2017); the detector antibody DC2E2 was prepared by biotinylation of DC2E2, whereby 120-fold excess of biotin, EZ-Link™ NHS-PEG4-Biotin (Thermo Scientific, #21329) over antibody concentration was used.

We prepared the pT217 calibrator as a synthetic peptide containing both epitopes of the DC2E7 and DC2E2 antibodies. This peptide was dissolved in a calibrator diluent at a concentration of 2 µg/ml, aliquoted and stored at –80 °C. The calibrator was diluted in a calibrator diluent (20 mM sodium phosphate pH 7.4, 137 mM NaCl, 2.7 mM KCl, 2 % BSA) in serial 1.6-fold dilutions starting from 2000 pg/mL, following by 1250, 781.25, 488.28, 305.18, 190.73, 119.21, 74.51 and 0 pg/ml. The prepared calibrator concentrations were mixed in a 3:1 ratio with a sample diluent (80 mM sodium phosphate pH 7.4, 548 mM NaCl, 10.8 mM KCl, 0.04 % casein, and 0.4 % Tween 20). The CSF samples were diluted with a sample diluent in the same way as described for the calibrator (3 volumes CSF + 1 volume sample diluent). The assay was performed as a 2-step assay 1.0 according to the manufacturer's recommendation (Quanterix).

Validation of the assay – experimental setup

The validation was conducted in an open-label fashion at the Department of Clinical Chemistry, Amsterdam, Netherlands. The protocol for p-tau T217 detection and quantitation was applied to Simoa using an HD-1 Analyzer. The assay's sensitivity, precision, linearity, parallelism, and recovery were analyzed as previously described¹⁰. To identify the lower limit of quantification (LLOQ), 16 blank samples (240 µl calibrator diluent + 80 µl sample diluent) were measured. The LLOQ (concentration) was based on a signal of 10

standard deviations above the mean of the 16 blank samples (using the calibration curve). Precision was determined by calculating the intra-assay, inter-assay and intra-plate reproducibility. For the dilution linearity, three different CSF samples were spiked with 3000 pg/ml of pT217 calibrator and diluted a 2-fold until below the theoretical LLOQ. To assess parallelism, five different CSF samples were diluted 2-fold. To determine sample recovery, four different CSF samples were spiked with low (200 pg/mL), medium (500 pg/mL) or high (1500 pg/mL) concentrations of the pT217 calibrator. PBS was spiked as a reference.

CLINICAL COHORTS

Participants

The first cohort from the ongoing Amsterdam Dementia Cohort included 88 subjects with age-matched controls, AD, non-fluent variant of primary progressive aphasia (nvPPA), semantic variant of PPA (svPPA), behavioural variant of frontotemporal dementia (bvFTD), primary progressive palsy (PSP) and corticobasal degeneration (CBD), Table 1. All subjects visited the memory clinic at VU University Medical Center Amsterdam (VUmc) for extensive clinical evaluations that consisted of neurological, physical and neuropsychological evaluations, biomarker analyses in CSF, electroencephalography and brain magnetic resonance imaging¹¹.

The second cohort included 44 cognitively normal elderly participants and 47 AD patients recruited from the Skåne University Hospital, Sweden. The inclusion criteria for the cognitively normal elderly participants were age ≥ 60 years, a Mini-Mental State Examination (MMSE) score of 28–30 points at the screening visit, absence of cognitive symptoms as evaluated by a physician, fluency in Swedish and not fulfilling the criteria of MCI or any dementia. Patients with AD were required to meet the criteria for probable AD as defined

by the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association¹².

The third cohort included patients from the Czech Brain Aging Study Plus Cohort. In total 11 patients with probable non-AD tauopathy were included: nvPPA (n = 5), PSP (n = 3) and CBD (n = 3). Patients with nvPPA fulfilled imaging criteria for nvPPA as was described by Gorno-Tempini *et al.*¹³. Patients with PSP were diagnosed as probable PSP with Richardson's syndrome¹⁴ and patients with CBD fulfilled the criteria for probable sporadic CBS¹⁵.

CSF sampling and core AD CSF biomarkers

CSF was handled in compliance with standard recommendations¹⁶. All the samples were collected and stored in polypropylene tubes at -80°C according to LP consensus protocols¹⁷. The A β 42, t-tau, p-tau T181 and A β 40 were measured by Fujirebio Innotech ELISA assays (Fujirebio, Tokyo, Japan). For the analysis of A β x-42/ A β x-40 ratio in cohort 2, a Meso Scale Discovery Abeta Triplex assay was used (Meso Scale Discovery, Rockville, MD). Cohorts 1 and 3 were analysed in Amsterdam and cohort 2 was analysed in Gothenburg. All analyses were conducted in an open-label fashion.

IMMUNOHISTOCHEMICAL STAINING

For immunohistochemistry, the following brain areas were used: hippocampus and entorhinal cortex from AD (Braak stage VI, n = 3), FTD (Pick's disease, n = 3), control brain (Braak stage I, n = 3) and prodromal AD (Braak stage III, n = 3), caudate nucleus from CBD (n = 3) and putamen/caudate nucleus from PSP (n = 3). The brain tissue paraffin blocks were obtained from the Amsterdam brain bank.

The brain blocks embedded in paraffin were cut on a microtome (Leica RM2255) to get 8 μ m thick sections. The sections were placed on HistoBond slides (Marienfeld, Germany). For immunohistochemistry sections were pre-treated with formic acid and heat (autoclave, 121°C, 20 min.), followed by overnight incubation with primary antibodies (AT8 1:1000, DC2E7 1:10 000, DC2E2 1:200). All sections were incubated with anti-mouse biotinylated secondary antibody at room temperature for 1 hour and with avidin-biotin peroxidase-complex for 1 hour. The immunoreaction was visualized with VIP (Vectastain Elite ABC Kit, Vector Laboratories, CA, USA) and counterstained with methyl green (Vector Laboratories).

STATISTICAL ANALYSIS

First, the performance of the diagnostic assay was evaluated based on diagnostic accuracy, positive predictive value, negative predictive value, sensitivity and specificity. False positive fraction ($1 - \text{specificity}$) and true positive fraction (sensitivity) were calculated. Based on this result area under the curve (AUC), 95% confidence interval for AUC and optimal threshold were calculated¹⁸. Where necessary, a linear approximation to calculate specificity and sensitivity for pre-specified thresholds was used. The equality of AUC curves using Wald test was also tested¹⁹. Second, the differences in means of p-tau T217 between AD and other diagnoses were assessed using a bootstrap two-sample *t*-test (the number of bootstrap samples was 10000) followed by Bonferroni multiple adjustment of p-values. The differences in means of p-tau T217 between AD and controls were assessed using a bootstrap two-sample *t*-test (number of bootstrap samples was 10000). Additionally, the effect size Cohen's *d* and its empirical Wald 95% CI was calculated using variance stabilizing transformation²⁰. Finally, the association between various CSF biomarkers was characterized by Pearson product-moment correlation coefficient and tested using Fisher Z-

test²¹. All alternative hypotheses were two-sided and statistical tests were performed at significance level equal to 0.05.

The primary research question

Does the p-tau T217 assay distinguish AD dementia from other dementias and healthy controls? This study provides Class III evidence that a CSF immunoassay for p-tau T217 distinguishes AD from other dementias and healthy controls.

Standard protocol approvals, registrations, and participant consents

Written informed consent was obtained from all patients (or guardians of patients) participating in the study. All protocols were approved by Ethical committees of Alzheimer Center, Amsterdam Neuroscience, Amsterdam, Netherlands; Lund University, Sweden and Motol University Hospital, Prague, Czech Republic.

Data availability

Anonymized data will be shared on request from qualified investigators.

RESULTS

Validation of the p-tau T217 ultrasensitive immunoassay for human CSF

LLOQ based on $n = 16$ blank values were calculated to be 184.4 pg/mL. The mean parallelism of all 5 samples was 86%. Two out of five CSF samples fell within the acceptable range (85–115%) and were thus parallel to the pT217 calibrator. The other three CSF samples were just outside the criteria of 85–115% (81, 82 and 76%). The mean recovery of three CSF samples was 116% (low: 110%; medium: 116%; high: 123%), just outside the predefined criteria of 85–115%, meaning that there was almost no difference between the sample matrix and calibrator diluent. The assay was linear between a 2- and 16-fold dilution and no hook effect was observed. The precision of the assay was determined by means of the repeatability (intra-assay; 3.0%), intermediate precision (inter-assay; 10.3%) and within-plate reproducibility (intra-plate; 3.4%). Data available from Dryad (Suppl. Table 1): <https://doi.org/10.5061/dryad.tdz08kpwz>. The results showed a robust performance of the assay. Overall, we concluded that the assay for p-tau T217 on the Simoa HD-1 Analyzer is suitable for the measurement of p-tau T217 in human CSF samples.

Monoclonal antibodies DC2E2 and DC2E7 recognize pathology in human Alzheimer's disease brain and tauopathies

Neither DC2E2 nor DC2E7 recognized normal tau in the hippocampus of healthy controls fulfilling criteria for Braak stage I (Fig. 1A). In patients in the prodromal stage of AD (Braak stage III), the antibodies identified neurofibrillary tangles, neuropil threads and neuritic plaques distributed mainly in the hippocampus and entorhinal and transentorhinal cortex

(Fig. 1B). Finally, in late stage AD (Braak stage VI), the antibodies recognized extensive tau pathology in the hippocampus (Fig. 1C) and other cortical areas.

In addition to AD (Fig. 2A,B, higher magnification M) both DC2E7 and DC2E2 recognized tau pathology in other tauopathies, including Pick's disease, CBD and PSP (Fig. 2DE higher magnification N, 2GH, higher magnification O, 2JK). The antibodies displayed the same staining pattern, the same type and load of tau pathology as monoclonal antibody AT8, which is considered to be the gold standard for histopathological staining²² (Fig. 2C,F,I,L).

The p-tau T217 ultrasensitive immunoassay differentiates AD from frontotemporal dementia

We measured p-tau T217 concentration in CSF from patients with nvPPA, svPPA, bvFTD, PSP and CBD (cohort 1,3; Fig. 3A). The p-tau T217 assay discriminated between AD and non-AD neurodegenerative disorders (cut-off 242 pg/ml, AUC 0.91 [95% CI (0.80,0.96)], with accuracy of 90%, with 78% PPV, 97% NPV, 88% specificity [95% CI (0.79,0.98)] and 93% sensitivity [95% CI (0.78,0.99)]) compared to p-tau T181 ELISA (Fig. 3B) (cut-off 52 pg/ml, AUC 0.94 [95% CI (0.84,0.98)] showing 78% accuracy, 58% PPV, 98% NPV, 71% specificity [95% CI (0.44,0.99)], 97% sensitivity [95% CI (0.89,0.99)]). There was no statistically significant difference in AUCs between p-tau T217 and p-tau T181 (p-value = 0.912). Data available from Dryad (Suppl. Fig.2): <https://doi.org/10.5061/dryad.tdz08kpwz>.

Comparison of means showed that the assay significantly differentiated between AD and nvPPA (p < 0.0001; Cohen's d = 1.433, 95%CI (0.758,2.171)), svPPA (p < 0.0001; Cohen's d = 1.469, 95%CI (0.714,2.296)), bvFTD (p < 0.0001; Cohen's d = 1.586, 95%CI (0.822,2.426)), PSP (p < 0.0001; Cohen's d = 1.745, 95%CI (1.105,2.455)), and CBD (p < 0.0001, Cohen's d = 1.452; 95%CI (0.79,2.177)).

The p-tau T217 ultrasensitive immunoassay differentiates AD from controls

Further, we aimed to prove the diagnostic value of the assay to distinguish individuals with AD from healthy individuals. The p-tau T217 immunoassay was used to analyze CSF samples from AD patients (n = 47) and control individuals (n = 44) (cohort 2). We found that the assay distinguished AD patients from healthy individuals with very high sensitivity and specificity (cut-off 162.8 pg/ml, AUC 0.98 [95% CI (0.91, 0.99)], sensitivity 98 % [95% CI (0.88,0.99)], specificity 93 % [95% CI (0.85,0.99)]; Fig.4A). In comparison, p-tau T181 ELISA (cut-off 60 pg/ml, AUC 0.98 [95% CI (0.91,0.99)]), currently considered one of the best biomarkers for AD, showed 98 % sensitivity [95% CI (0.88,0.99)] and 86 % specificity [95% CI (0.76,0.99)]; (Fig. 4B). There was no statistically significant difference in AUCs between p-tau T217 and p-tau T181 ($p = 0.574$). Mean comparison showed that the p-tau T217 assay significantly differentiated between AD and controls ($p < 0.0001$; Cohen's $d = 2.160$, 95% CI (1.663,2.701)). All other commonly used CSF biomarkers for AD, t-tau, A β 42, A β 42/A β 40 ratio and also p-tau T217/t-tau ratio, showed worse diagnostic performance than p-tau pT217 alone (Fig.4C–F).

The amount of p-tau T217 correlates with that of p-tau T181, t-tau and A β 40 but not with A β 42

In the second cohort, we found a strong correlation between p-tau T217 and p-tau T181 (Fig.5A, $r = 0.941$, 95% CI (0.896,0.967), $p < 0.0001$) and between p-tau T217 and t-tau (Fig.5B, $r = 0.902$, 95% CI (0.829,0.944), $p < 0.0001$) in AD. Interestingly, we observed a

correlation between p-tau T217 and A β 40 (Fig. 5C, $r = 0.617$, 95% CI (0.402,0.768), $p < 0.0001$) but not between p-tau T217 and A β 42 (Fig.5D, $r = 0.131$, 95% CI (-0.162,0.403), $p = 0.380$) in AD. The correlation between p-tau T217 and other tau CSF biomarkers was weaker in healthy individuals; between p-tau T217 and p-tau T181 (Fig.5A, $r = 0.787$, 95% CI (0.640,0.879), $p < 0.0001$), and between p-tau T217 and t-tau (Fig.5B, $r = 0.541$, 95% CI (0.290,0.722), $p = 0.0001$). We did not find any correlations between p-tau T217 and A β 40 (Fig. 5C, $r = 0.235$, 95% CI (-0.066,0.497), $p = 0.125$) and between p-tau T217 and A β 42 (Fig.5D, $r = -0.126$, 95% CI (-0.407,0.178), $p = 0.419$) in healthy individuals.

DISCUSSION

Previous studies have demonstrated that CSF p-tau T217 could potentially be a key biomarker to monitor tau pathology in AD pathophysiology, and that its role might differ from other p-tau biomarkers^{6,7}. In this study we introduced a novel immunoassay, detecting p-tau T217 in CSF, which is based on the highly sensitive Simoa technology. Our results show that the assay discriminates between AD and other neurodegenerative dementias with high specificity and sensitivity, and demonstrates better diagnostic accuracy than p-tau T181 assay. This is in line with recent study on the new CSF p-tau T217 assay based on MSD (Meso Scale Discovery) platform, where the authors showed better diagnostic performance of the assay when comparing with p-tau T181²³. Moreover, the same study revealed that the correlations with tau PET tracer [18F]flortaucipir were consistently higher for p-tau217 than p-tau181 and that [18F]flortaucipir retention was more related to longitudinal changes in p-tau217 than in p-tau181. Similarly, by using quantitative mass spectrometry approach, Barthelemy et al. (2020) demonstrated that pT217 differentiated between Alzheimer's disease patients and other neurodegenerative

diseases with higher specificity and sensitivity than pT181²⁴. The better discriminatory potency of p-tau T217 for AD might be attributed to its ability to reflect both amyloid and tau pathological pathways. It has been reported, that increased p-tau T217 levels in CSF are related to the brain amyloid load positive participants already at the preclinical stage what further supports that this biomarker is AD- specific⁶. The specificity of T217 tau phosphorylation change for AD surpasses other phosphorylated tau sites such as T181, S199, S202 and T205⁶. These data suggest that p-tau T217 represents a promising AD biomarker.

The assay also distinguishes AD patients from age-matched healthy subjects, which is in agreement with mass spectrometry data on p-tau T217²⁴. Low levels of p-tau T217 in healthy controls could be caused by rapid degradation (or de-phosphorylation) of tau species phosphorylated at this particular position. Indeed, one study has demonstrated that phosphorylation of tau on T217 had the most robust effect on shortening tau half-life in physiological conditions⁸.

When investigating the relationship between the different biomarkers in AD patients, p-tau T217 showed a strong correlation with t-tau, p-tau T181 and A β 40, but not with A β 42. It was hypothesized that A β 42 is toxic to neurons, while A β 40 is more strongly associated with progressive neuronal degeneration²⁵. The strong correlation between p-tau T217, t-tau, p-tau T181 and A β 40 suggest that these proteins may be released from neurons in a coordinated fashion, perhaps in relation to neuronal activity, as has been previously suggested^{26, 27}.

Interestingly, our p-tau T217 assay discriminates AD from FTD, despite the fact that p-tau T217 was present in brain tissues of both AD and FTD patients. The p-tau T217 species were found to be present in all types of neurofibrillary lesions (neurofibrillary tangles, neuropil

threads, dystrophic neurites and neuritic plaques), but also in glial tau pathology (PSP, CBD) and in Pick's bodies (FTD). Although our findings indicate that p-tau T217 species are involved in the developing tau pathology in neurons and glial cells in various human tauopathies, the CSF levels of p-tau T217 species are elevated almost exclusively in AD. Among the potential limitations of our study is the small sample size, results should be replicated in larger cohorts ideally characterised by both amyloid and tau PET imaging and validated in routine clinical practice. In order to introduce p-tau T217 assay in clinical routine practice, the technology should undergo a structured assessment to evaluate its benefit in terms of clinical utility and cost-effectiveness. Our novel immunoassay for quantification of p-tau T217 in the CSF demonstrates that the assay is highly specific for AD and seems superior to the p-tau T181 assay in the AD diagnostic classifications. In the future, the assay can potentially be used for diagnostic purposes as well as for patient stratification and enrichment of target population in clinical trials for disease-modifying therapies.

APPENDIX AUTHORS

Name	Location	Contribution
Jozef Hanes	AXON Neuroscience R&D Services SE, Bratislava, Slovakia	Design, data acquisition and analysis, drafted the manuscript for intellectual content
Andrej Kovac	AXON Neuroscience R&D Services SE, Bratislava, Slovakia	Interpreted the data; revised the manuscript for intellectual content
Hlin Kvartsberg	Sahlgrenska University Hospital, Mölndal, Sweden	Design, data acquisition and analysis, drafted the manuscript for intellectual content
Eva Kontsekova	AXON Neuroscience R&D Services SE, Bratislava, Slovakia	Interpreted the data; revised the manuscript for intellectual content
Lubica Fialova	AXON Neuroscience R&D Services SE, Bratislava, Slovakia	Data acquisition and analysis, revised the manuscript for intellectual content
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		revised the manuscript for intellectual content
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Table 1 – Characteristics of the study cohorts

AMSTERDAM DEMENTIA COHORT						
	Age	Age	Males	Number	MMSE	MMSE
	average	St Dev	(%)		average	St Dev
AD	68.0	7.5	26.7	30	18.3	6.0
bvFTD	62.3	10.8	40	10	26.1	3.5
nvPPA	69.6	6.1	53.3	10	23.0	6.6
svPPA	64.1	2.8	53.3	10	24.4	4.9
PSP	70.5	4.2	50	16	25.1	3.0
CBD	69.2	7.1	75	12	23.5	3.6
SKÅNE UNIVERSITY HOSPITAL						
AD	74.4	7.2	42	47	19.0	4.8
Age-matched controls	73.6	5.6	42	44	29.1	0.8
CZECH BRAIN AGING STUDY PLUS COHORT						
nvPPA	21.8	5.8	40	5	24.4	4.4
PSP	74.7	8.1	33.3	3	23.3	6.7
CBD	67	5.3	33.3	3	17.3	4.5

FIGURE LEGEND

Figure 1

Monoclonal antibodies DC2E2 and DC2E7 do not recognize tau in a normal brain (A, Braak stage I). In the prodromal stage (B, Braak stage III) and full-blown AD (C, Braak stage VI) both antibodies identify neurofibrillary pathology. They stain neurofibrillary tangles, neuropil threads and neuritic plaques. Tool bar = 100 μ m.

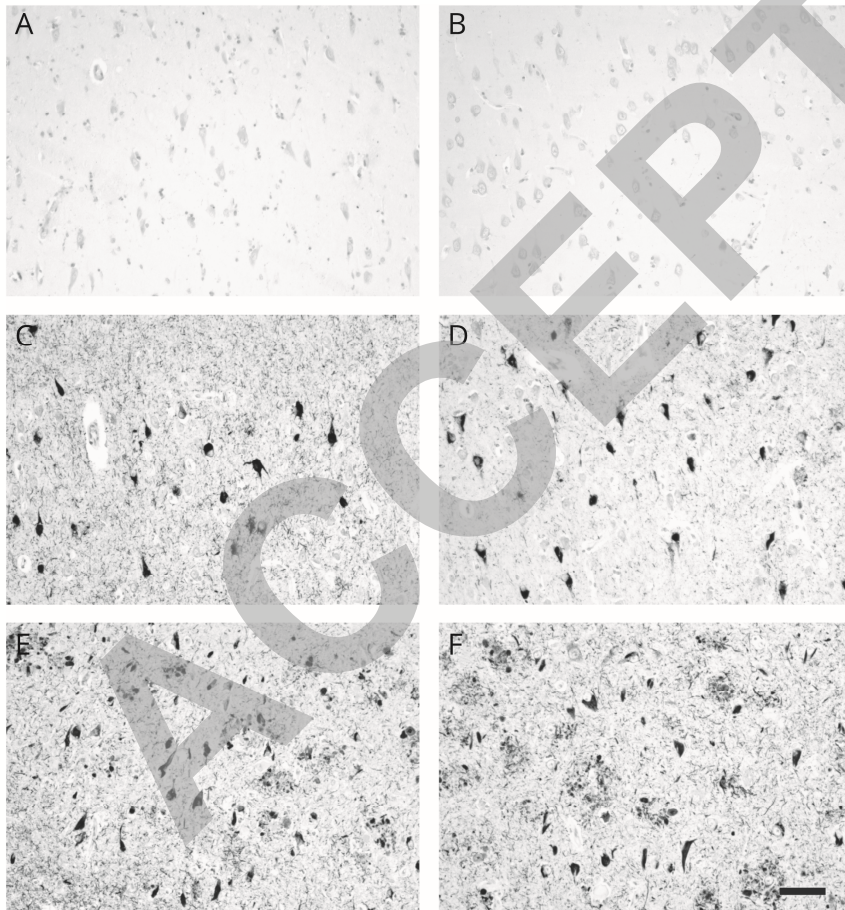


Figure 2

Monoclonal antibodies DC2E7 and DC2E2 recognized neurofibrillary pathology in the hippocampus of AD patients (A, B), Pick's bodies in the dentate gyrus of FTD patients (D, E), glial tau pathology in caudate nucleus of patients suffering either from CBD (G, H) and PSP (J, K). AT8 was used as a control for histopathological staining (C, F, I, L). Higher magnification of tau pathology in AD (M), Pick's disease (N) and CBD (O). Tool bar = 200 μ m (A-L), 50 μ m (M-O).

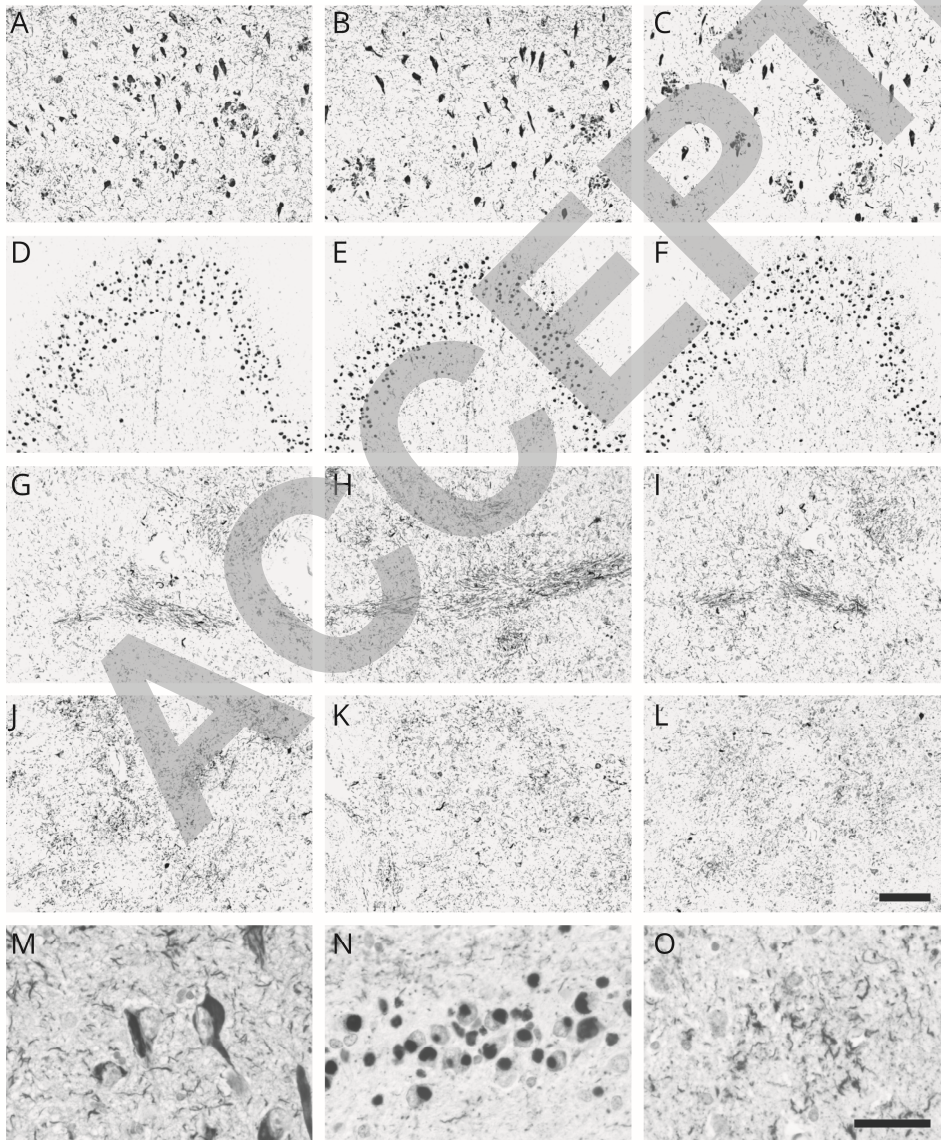


Figure 3

CSF p-tau T217 levels (A) and p-tau T181 in AD, behavioral variant of frontotemporal dementia (bvFTD), non-fluent variant of primary progressive aphasia (nvPPA), semantic variant of primary progressive aphasia (svPPA), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD). The lines indicate cut-off values (p-tau T217, 242 pg/ml; p-tau T181, 184pg/ml). CSF samples obtained from cohort 1 and 3.

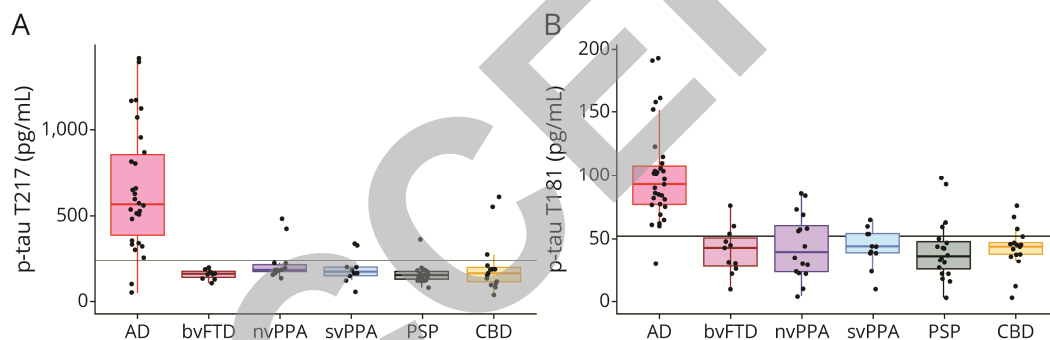


Figure 4

The level of p-tau T217 (A), p-tau T181 (B), t-tau (C), ratio p-tau T217/t-tau (D), amyloid β 42 (E) and ratio amyloid β 42/40 (F) in AD and control subjects. CSF samples obtained from cohort 2.

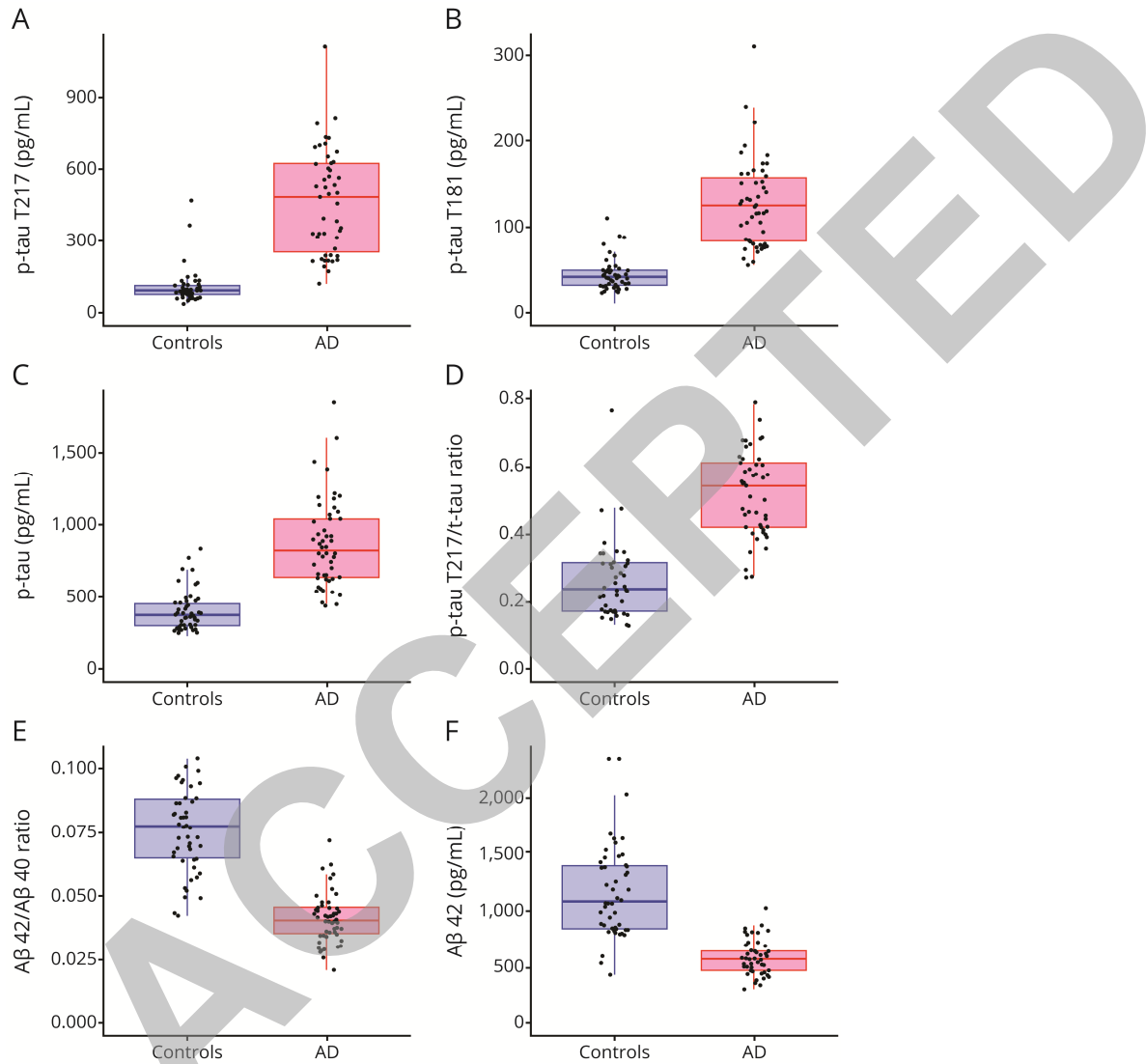
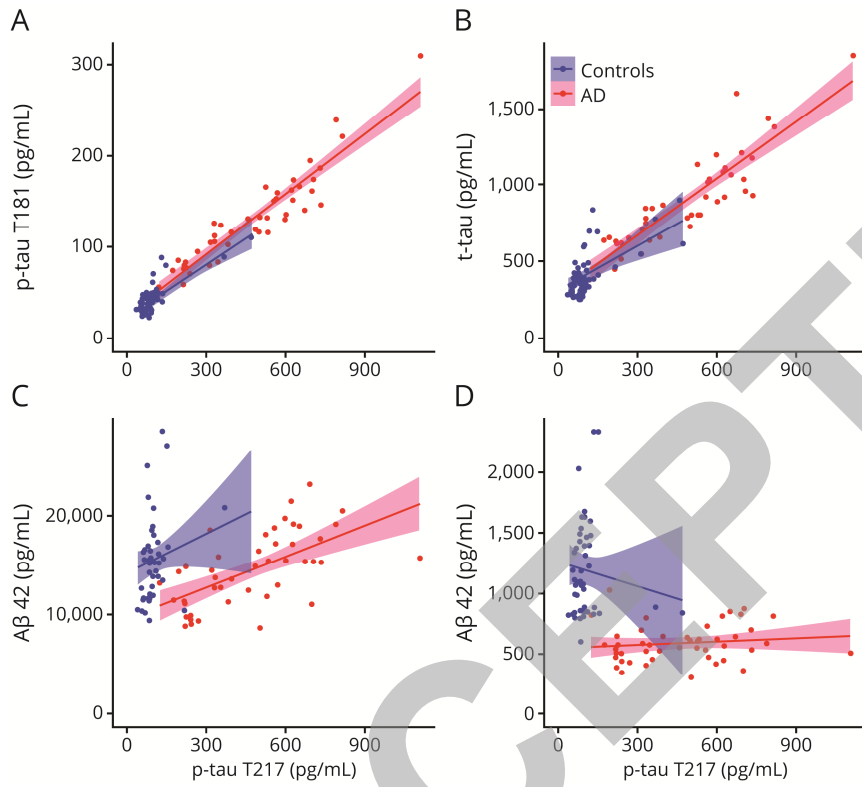


Figure 5

The correlation between p-tau T217 and p-tau T181 (A), t-tau (B), A β 40 (C) and A β 42 (D) in AD in healthy subjects. CSF samples obtained from cohort 2.



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