

Mass spectrometric simultaneous quantification of tau species in plasma shows differential associations with amyloid and tau pathologies

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Supplementary Table 1. Plasma levels and demographics of participants in Cohort 1.

Biomarker values and age are given in median fmol/mL (IQR) and mean (SD). Units: plasma biomarkers (fmol/mL), CSF biomarkers (pg/mL) and age (years). Abbreviations: AD (Alzheimer's disease), MCI (Mild-Cognitively Impaired), F (female), IQR (Interquartile Range), SD (Standard Deviation).

		Control (N=23)	AD MCI (N=24)	AD (N=27)	MCI other (N=50)	Other dementia (N=33)
P-tau181	Median (IQR)	0.0272 (0.0131)	0.0407 (0.0146)	0.0426 (0.0115)	0.0299 (0.0161)	0.0304 (0.0126)
	Mean (SD)	0.0274 (0.00977)	0.0458 (0.0189)	0.0454 (0.0171)	0.0316 (0.0121)	0.0333 (0.0154)
Tau 212-221	Median (IQR)	0.221(0.0549)	0.288(0.119)	0.283(0.137)	0.252(0.146)	0.250(0.112)
	Mean (SD)	0.224(0.0922)	0.314(0.0969)	0.298(0.118)	0.270(0.122)	0.259(0.105)
P-tau217	Median (IQR)	0.00213 (0.00213)	0.00553 (0.00418)	0.00870 (0.00775)	0.00245 (0.00239)	0.00260 (0.00255)
	Mean (SD)	0.00388 (0.00494)	0.00694 (0.00602)	0.0104 (0.00674)	0.00350 (0.00292)	0.00442 (0.00558)
P-tau199	Median (IQR)	0.00285 (0.00543)	0.00255 (0.00475)	0.00390 (0.00475)	0.00110 (0.00360)	0.00140 (0.00380)
	Mean (SD)	0.00372 (0.00446)	0.00687 (0.0159)	0.00382 (0.00350)	0.00208 (0.00242)	0.00222 (0.00275)
P-tau202	Median (IQR)	0.0397 (0.0385)	0.0702 (0.0788)	0.0469 (0.0420)	0.0334 (0.0337)	0.0451 (0.0211)
	Mean (SD)	0.0488 (0.0416)	0.0827 (0.0633)	0.0623 (0.0438)	0.0472 (0.0439)	0.0402 (0.0205)
P-tau205	Median (IQR)	0 (0.000115)	0.000590 (0.00216)	0.00137 (0.00191)	0(0.000843)	0.000300 (0.000910)
	Mean (SD)	0.00167 (0.00545)	0.00113 (0.00132)	0.00201 (0.00220)	0.00193 (0.00760)	0.00102 (0.00260)
P-tau231	Median (IQR)	0.00340 (0.00363)	0.00885 (0.0127)	0.0166 (0.0155)	0.00470 (0.00770)	0.00410 (0.00780)
	Mean (SD)	0.00477 (0.00476)	0.0129 (0.0121)	0.0164 (0.0104)	0.00593 (0.00581)	0.00756 (0.0101)
CSF Aβ42/40	Median (IQR)	6.49 (2.33)	16.11 (5.57)	15.39 (5.54)	9.58 (3.80)	8.46 (3.76)
	Mean (SD)	7.46 (2.41)	17.31 (5.19)	16.96 (3.91)	9.54 (2.63)	8.93 (2.73)
CSF p-tau	Median (IQR)	13.30 (4.00)	24.90 (19.48)	26.77 (17.72)	14.10 (9.70)	17.80 (10.80)
	Mean (SD)	13.45 (2.65)	29.62 (22.97)	40.50 (37.40)	16.53 (6.95)	21.39 (15.01)
CSF t-tau	Median (IQR)	159.00 (46.24)	241.18 (173.86)	321.00 (208.00)	165.50 (94.33)	217.00 (127.00)
	Mean (SD)	165.81 (29.01)	301.69 (200.19)	661.86 (1503.33)	192.94 (71.97)	263.40 (170.75)
MMSE	Median (IQR)	27.00 (4.00)	27.00 (2.25)	19.00 (5.50)	25.00 (3.75)	23.00 (7.00)
	Mean (SD)	27.17 (2.18)	27.08 (2.20)	18.63 (4.42)	24.36 (4.10)	21.27 (5.18)
Age	Median (IQR)	63.00 (12.00)	72.50 (10.25)	68.00 (13.50)	66.00 (10.75)	70.00 (9.00)
	Mean (SD)	62.26 (8.52)	72.25 (8.28)	68.93 (9.19)	66.14 (8.34)	69.61 (7.24)
Sex (F)		13 (56.5%)	13 (54.2%)	19 (70.4%)	34 (68.0%)	17 (51.5%)

Supplementary Table 2. Demographics, plasma and imaging biomarker levels of participants in Cohort 2.

Biomarker values and age are given in median (IQR) and mean (SD). Units: plasma biomarkers (fmol/ml), imaging biomarkers (SUVR) and age (years). Abbreviations: CU- (Cognitively Unimpaired amyloid PET negative), CU+ (Cognitively Unimpaired amyloid PET positive), CI+ (Cognitively Impaired amyloid PET positive) and CI- (Cognitively Impaired amyloid PET negative), F (female), SUVR (Standard uptake value ratio), IQR (Interquartile Range), SD (Standard Deviation).

		CU- (N=18)	CU+ (N=8)	CI+ (N=18)	CI- (N=7)
P-tau181	Median (IQR)	0.0431(0.0172)	0.0585(0.0290)	0.0585(0.0440)	0.0611(0.0314)
	Mean (SD)	0.0469(0.0170)	0.0580(0.0205)	0.0661(0.0291)	0.0671(0.0425)
Tau 212-221	Median (IQR)	0.467(0.142)	0.583(0.280)	0.451(0.256)	0.541(0.0843)
	Mean (SD)	0.495(0.141)	0.551(0.200)	0.488(0.229)	0.527(0.125)
P-tau217	Median (IQR)	0.00285(0.00268)	0.00758(0.00609)	0.0141(0.0181)	0.00600(0.00423)
	Mean (SD)	0.00361(0.00223)	0.00739(0.00424)	0.0167(0.0109)	0.00506(0.00294)
Tau 195-209	Median (IQR)	0.625(0.176)	0.778(0.379)	0.714(0.384)	0.631(0.108)
	Mean (SD)	0.635(0.141)	0.724(0.267)	0.717(0.347)	0.644(0.139)
P-tau199	Median (IQR)	0.00595(0.00340)	0.00650(0.00560)	0.00600(0.00458)	0.00860(0.00335)
	Mean (SD)	0.00579(0.00275)	0.00813(0.00435)	0.00692(0.00425)	0.00856(0.00309)
P-tau202	Median (IQR)	0.0125(0.00339)	0.0139(0.00943)	0.0126(0.00600)	0.0145(0.00458)
	Mean (SD)	0.0127(0.00431)	0.0157(0.00653)	0.0130(0.00666)	0.0156(0.00415)
P-tau205	Median (IQR)	0.000315(0.000430)	0.000890(0.000698)	0.00206(0.00278)	0.000870(0.000585)
	Mean (SD)	0.000451(0.000356)	0.000904(0.000650)	0.00199(0.00160)	0.000867(0.000585)
P-tau231	Median (IQR)	0.00785(0.00915)	0.0215(0.0135)	0.0247(0.0288)	0.0150(0.0118)
	Mean (SD)	0.0105(0.00804)	0.0187(0.00928)	0.0288(0.0182)	0.0192(0.0172)
Tau PET	Median (IQR)	0.817(0.140)	0.845(0.183)	1.97(1.48)	0.826(0.144)
	Mean (SD)	0.828(0.0887)	0.885(0.131)	1.92(0.792)	0.840(0.124)
	Missing	1 (5.6%)	0 (0%)	0 (0%)	0 (0%)
Amyloid PET	Median (IQR)	1.20(0.183)	1.88(0.205)	2.53(0.318)	1.37(0.165)
	Mean (SD)	1.26(0.122)	1.95(0.326)	2.53(0.443)	1.40(0.177)
Sex	female	9 (50.0%)	4 (50.0%)	9 (50.0%)	4 (57.1%)
	male	9 (50.0%)	4 (50.0%)	9 (50.0%)	3 (42.9%)
Age	Median (IQR)	66.3(47.4)	72.2(4.03)	72.2(9.56)	76.5(3.54)
	Mean (SD)	54.6(24.1)	72.3(2.86)	71.5(5.99)	75.7(5.24)

Supplementary Table 3. Plasma biomarker levels in non-AD participants in Cohort 2. Biomarker values are given in median (IQR) and mean (SD) (fmol/mL). Abbreviations: FTD, Frontotemporal Dementia), PSP, (Progressive Supranuclear Palsy), IQR (Interquartile Range), SD (Standard Deviation).

		FTD (N=4)	PSP (N=2)
P-tau181	Median (IQR)	0.0345(0.0225)	0.0853(0.0270)
	Mean (SD)	0.0534(0.0392)	0.0853(0.0382)
Tau 212-221	Median (IQR)	0.476(0.109)	0.603(0.139)
	Mean (SD)	0.466(0.0785)	0.603(0.196)
P-tau217	Median (IQR)	0.00228(0.00184)	0.00370(0.000600)
	Mean (SD)	0.00259(0.00141)	0.00370(0.000849)
Tau 195-209	Median (IQR)	0.556(0.162)	0.737(0.123)
	Mean (SD)	0.553(0.110)	0.737(0.174)
P-tau199	Median (IQR)	0.00555(0.000475)	0.00930(0.00210)
	Mean (SD)	0.00593(0.000785)	0.00930(0.00297)
P-tau202	Median (IQR)	0.0131(0.00246)	0.0177(0.00338)
	Mean (SD)	0.0145(0.00436)	0.0177(0.00477)
P-tau205	Median (IQR)	0.000245(0.0000500)	0.000850(0.000430)
	Mean (SD)	0.000220(0.0000753)	0.000850(0.000608)
P-tau231	Median (IQR)	0.00705(0.00455)	0.0201(0.00805)
	Mean (SD)	0.00950(0.00609)	0.0201(0.0114)

Supplementary Table 4. Correlations between biomarkers.

Cross-correlation matrix showing Spearman rho correlation values between plasmatic and imaging biomarkers. Significant correlations are indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (two-sided).

	pTau217	pTau199	pTau202	pTau181	pTau205	pTau231	Tau212-221	Tau195-209	Tau PET	Amyloid PET
pTau217	1									
pTau199	0.46***	1								
pTau202	0.49***	0.87***	1							
pTau181	0.72***	0.68***	0.81***	1						
pTau205	0.82***	0.66***	0.70***	0.80***	1					
pTau231	0.86***	0.68***	0.73***	0.90***	0.89***	1				
Tau212-221	0.55***	0.78***	0.86***	0.75***	0.74***	0.70***	1			
Tau195-209	0.70***	0.69***	0.81***	0.80***	0.81***	0.78***	0.95***	1		
Tau PET	0.58***	0.10	0.07	0.24	0.49***	0.43**	0.09	0.23	1	
Amyloid PET	0.70***	0.13	0.17	0.42**	0.52***	0.60***	0.13	0.29*	0.68***	1

Supplementary Table 5. Biomarker performance.

The area under the Receiver Operating characteristic curves (AUC-ROC) and the 95% confidence intervals are reported below to indicate the ability of plasma p-tau181, p-tau205, p-tau217 and p-tau231 to distinguish participants grouped according to their amyloid PET status or Braak stages.

	A β PET positive vs negative	Braak I-IV vs Braak V-VI
pTau181	0.65 95% CI: 0.50-0.81	0.64 95% CI: 0.40-0.87
pTau205	0.72 95% CI: 0.57-0.86	0.81 95% CI: 0.58-1
pTau205/Tau195-209	0.75 95% CI: 0.61-0.89	0.84 95% CI: 0.63-1
pTau217	0.85 95% CI: 0.74–0.95	0.81 95% CI: 0.59-1
pTau217/Tau212-221	0.94 95% CI: 0.87-1	0.96 95% CI: 0.9-1
pTau231	0.76 95% CI: 0.63–0.90	0.72 95% CI: 0.48-0.96

Supplementary Table 6. Tryptic tau endogenous peptides targeted in the study.

Respective dominant charge state, monoisotopic m/z value, optimal normalized collision energy for higher energy collision-induced dissociation (NCE) and optimal compensation voltage (CV) used for FAIMS for each peptide are shown.

Phospho site	Peptide aa positions	Target peptide sequence	Charge state	m/z	CE [%]	FAIMS CV (V)
T181	175-190	TPPAPK[pT]PPSSGEPPK	3	556.6062	25	-70
-	195-209	SGYSSPGSPGTPGSR	2	697.321	25	-50
S199	195-209	SGYS[pS]PGSPGTPGSR	2	737.3039	25	-50
S202	195-209	SGYSSPG[pS]PGTPGSR	2	737.3039	25	-50
T205	195-209	SGYSSPGSPG[pT]PGSR	2	737.3039	25	-50
-	212-221	TPSLPTPPTTR	2	533.798	25	-70
T217	212-221	TPSLP[pT]PPTR	2	573.781	27	-60
T231	225-240	KVAVVR[pT]PPKSPSSAK	3	577.9887	30	-60

Supplementary Table 7. Heavy standards used for the normalization of quantified peptides.

Respective monoisotopic m/z value, heavy labelling and spiked in concentrations are shown for each peptide. The heavy labeled aminoacids are marked in bold. Dominant charge state, optimal normalized collision energy for higher energy collision-induced dissociation (CE) and optimal compensation voltage used for FAIMS are the same as for the corresponding light peptides shown in Supplementary Table 6.

Phospho site	Peptide aa positions	Target peptide sequence	m/z	Spiked in per sample (fmol)
T181	175-190	TPPAPK[pT]PPSSGEPPK	558.6129	1
-	195-209	SGYSSPGSPGTPGSR	702.325	0.5
S199	195-209	SGYS[pS]PGSPGTPGSR	742.3175	1
S202	195-209	SGYSSPG[pS]PGTPGSR	742.3081	0.5
T205	195-209	SGYSSPGSPG[pT]PGSR	742.3175	0.1
-	212-221	TPSLTPPTR	538.802	1
T217	212-221	TPSLP[pT]PPTR	578.786	0.5
T231	225-240	KVAVVR[pT]PPKSPSSAK	582.0022	1

Supplementary Table 8. Coefficient of variation (CV) of each targeted peptide.

Ratio to standard values for each Quality Control (QC) sample (n=8). QCs samples were generated by pooling plasma from healthy individuals and aliquoting in 1ml-volume samples. CVs were calculated as the standard deviation divided by the mean and expressed in percentage (%).

	p181	195-209	p199	p202	p205	212-221	p217	p231
QC1	0.1470	4.6961	0.0409	0.1229	0.0352	2.0081	0.0293	0.0868
QC2	0.1331	4.0255	0.0324	0.1019	0.0373	1.7325	0.0228	0.0798
QC3	0.1272	4.1781	0.0266	0.0986	0.0355	1.7186	0.0244	0.0737
QC4	0.1455	4.3311	0.0363	0.1183	0.0408	1.8975	0.0258	0.0784
QC5	0.1482	4.6931	0.0315	0.0988	0.0451	1.9108	0.0261	0.0800
QC6	0.1411	4.4279	0.0358	0.1052	0.0425	1.9529	0.0242	0.0935
QC7	0.1409	4.8416	0.0290	0.1074	0.0413	2.3541	0.0304	0.0792
QC8	0.1361	4.7877	0.0285	0.0956	0.0469	1.9765	0.0243	0.0677
CV (%)	5.23%	6.71%	14.65%	9.23%	10.63%	10.14%	10.23%	9.73%

Supplementary Table 9. Fold-changes in the levels of each phosphorylated peptide in the Alzheimer's disease group compared to controls (Discovery cohort, H70 studies) alone or the ratio of the phosphorylated peptide vs non-phosphorylated.

	phospho peptide	phospho/195-209	phospho/212-221
p-tau181	1.61	1.19	0.78
p-tau199	1.17	0.88	0.78
p-tau202	1.02	0.78	0.69
p-tau205	4.05	3.37	2.72
p-tau217	1.97	1.45	1.36
p-tau231	2.76	2.07	2.07