**Supplementary Table 1.** List of the peptides generated from the tryptic digestion of the Tau 441 isoform

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Peptide Sequence | Residues | Isoforms | Detected in recombinant Tau (4000 pg/mL) | Detected in CSF pools |
|
|
| QEFEVMox2EDHAGTYGLGDR | 6 | - | 23 | all | yes | yes |
| DQGGYTMox2HQDQEGDTDAGLK | 25 | - | 44 | all | yes | yes |
| ESPLQTPTEDGSEEPGSETSDAK | 45 | - | 67 | 1N+2N | yes | yes |
| QAAAQPHTEIPEGTTAEEAGIGDTPSLED EAAGHVTQAR  | 68 | - | 87 | 2N | yes | yes |
| STPTAEDVTAPLVDEGAPGK  | 88 | - | 126 | 2N | yes | yes |
| GAAPPGQK  | 156 | - | 163 | all | yes | yes |
| TPPAPK | 175 | - | 180 | all | yes | yes |
| TPPSSGEPPK | 181 | - | 190 | all | yes | yes |
| SGYSSPGSPGTPGSR | 195 | - | 209 | all | yes | yes |
| TPSLPTPPTREPK | 212 | - | 224 | all | yes | yes |
| TPSLPTPPTR | 212 | - | 221 | all | yes | yes |
| LQTAPVPMox2PDLK | 243 | - | 254 | all | yes | yes |
| IGSTENLK | 260 | - | 267 | all | yes | yes |
| VQIINK | 275 | - | 280 | 4R | yes | yes |
| KLDLSNVQSK | 281 | - | 290 | 4R | yes | no |
| LDLSNVQSK | 282 | - | 290 | 4R | yes | no |
| HVPGGGSVQIVYKPVDLSK | 299 | - | 317 | 4R | yes | yes |
| SLGNIHHKPGGGQVE | 322 | - | 340 | all | yes | no |
| IGSLDNITHVPGGGNK | 354 | - | 369 | all | yes | yes |
| TDHGAEIVYK | 386 | - | 395 | all | yes | yes |
| SPVVSGDTSPR | 396 | - | 406 | all | yes | yes |
| HLSNVSSTGSIDMox2VDSPQLATLADE VSASLAK  | 406 | - | 438 | all | yes | no |

The following Tau theoretical peptides generated from the recombinant protein were not selected for detection in relation with their size/expected LC separation:GADGK, IATPR, GQANATR, IPAK, SGDR, VAVVR, TPPK, SPSSAK, HQPGGGK, CGSK, DNIK, VTSK, LDFK, VQSK, IETHK, LTFR, ENAK.

**Supplementary Table 2.** Demographic table

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **AD (n=10)** | **PSP (n=5)** | **DLB (n=4)** | **Non-AD (n=12)** | **ANOVA Significance level** |
|  | **Median** | **25 - 75 P** | **Median** | **25 - 75 P** | **Median** | **25 - 75 P** | **Median** | **25 - 75 P** |
| **Age** (years) | 79.6 | 69.9 to 83.3 | 66.8 | 55.5 to 74.9 | 73.15 | 62.8 to 81.0 | 69 | 57.8 to 78.2 | 0.675 |
| **MMSE** (/30) | 17.5 | 13.0 to 21.0 | 19.5 | 17 to 22 | 12.5 | 6.5 to 16.0 | 21 | 16.0 to 29.5 | 0.374 |
| **Aβ1-42**(pg/mL) | 319 | 311 to 377 | 486.5 | 374 to 636 | 504 | 327 to 764 | 628 | 531 to 737 | 0.152 |
| **Tau**(pg/mL) | 637 | 442 to 856 | 197.5 | 139 to 258 | 204.5 | 183 to 246 | 179 | 113 to 244 | < 0.001 |
| **p-Tau(181)**(pg/mL) | 86 | 83 to 118 | 26 | 17 to 41 | 43 | 37 to 48 | 34 | 26 to 43 | < 0.001 |

Median and quartiles of demographic data, Mini-Mental State Examination score (MMSE) and CSF biomarker levels (Aβ1-42, Tau, and p-Tau(181)) for the different clinical groups: Alzheimer’s disease (AD), progressive supranuclear palsy (PSP), dementia with Lewy body (DLB) and control patients (CTRL). One-way ANOVA test was performed between the four groups.

**Supplementary Table 3.** MS values of the different peptides in the different clinical groups (median and 25-75 percentile, pg/mL of equivalent recombinant tau) in the different clinical groups. The Mann-Whitney test is used to test the significance of the difference between two independent samples. It is the alternative for the Independent samples t-test, when the distribution of the samples is not Normal. The Mann-Whitney test combines and ranks the data from sample 1 and sample 2 and calculates a statistic on the difference between the sum of the ranks of sample 1 and sample 2. If the resulting p-value is small (p<0.05) then a statistically significant difference between the two samples can be accepted.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **AD (n=10)** | **PSP (n=5)** | **LBD (n=4)** | **Non-AD (n=12)** | **AD versus non-AD** |
|  | **Median** | **25 - 75 P** | **Median** | **25 - 75 P** | **Median** | **25 - 75 P** | **Median** | **25 - 75 P** | Mann-Whitney test |
| **pep\_6\_23** | 1.87 | 1.51 to 2.78 | 0.93 | 0.69 to 1.11 | 0.98 | 0.91 to 1.06 | 0.72 | 0.63 to 0.85 | p = 0.0001 |
| **pep\_25\_44** | 1.54 | 1.26 to 3.51 | 0.75 | 0.01 to 1.17 | 0.01 | 0.01 to 0.65 | 0.74 | 0.01 to 0.98 | p = 0.0032 |
| **pep\_45\_67** | 1.37 | 1.09 to 1.98 | 0.94 | 0.72 to 1.12 | 0.82 | 0.80 to 1.04 | 0.83 | 0.66 to 0.93 | p = 0.0002 |
| **pep\_68\_87** | 0.21 | 0.17 to 0.29 | 0.17 | 0.15 to 0.17 | 0.16 | 0.15 to 0.17 | 0.15 | 0.14 to 0.16 | p = 0.0035 |
| **pep\_88\_126** | 0.16 | 0.13 to 0.18 | 0.12 | 0.09 to 0.14 | 0.12 | 0.12 to 0.12 | 0.12 | 0.12 to 0.12 | p = 0.0001 |
| **pep\_156\_163** | 14.57 | 11.00 to 17.8 | 6.29 | 4.51 to 7.97 | 6.41 | 6.10 to 6.98 | 5.03 | 3.99 to 7.04 | p = 0.0001 |
| **pep\_175\_180** | 11.27 | 8.20 to 13.16 | 5.82 | 4.75 to 6.86 | 6.19 | 5.22 to 6.39 | 5.31 | 4.11 to 6.55 | p = 0.0002 |
| **pep\_181\_190** | 14.42 | 9.24 to 16.27 | 6.05 | 5.04 to 6.70 | 6.22 | 5.24 to 6.90 | 6.38 | 4.58 to 7.00 | p = 0.0002 |
| **pep\_195\_209** | 14.13 | 11.14 to 20.46 | 5.20 | 2.82 to 6.09 | 4.38 | 4.09 to 4.90 | 3.62 | 2.47 to 6.07 | p = 0.0001 |
| **pep\_212\_221** | 6.12 | 4.38 to 7.01 | 2.04 | 1.46 to 2.55 | 1.92 | 1.31 to 2.29 | 1.72 | 1.41 to 2.25 | p = 0.0001 |
| **pep\_212\_224** | 6.12 | 4.48 to 7.07 | 2.33 | 1.15 to 2.90 | 1.96 | 1.88 to 2.12 | 1.64 | 1.20 to 2.25 | p = 0.0001 |
| **pep\_243\_254** | 1.53 | 1.20 to 1.84 | 0.43 | 0.25 to 0.46 | 0.40 | 0.26 to 0.57 | 0.32 | 0.28 to 0.36 | p = 0.0001 |
| **pep\_260\_267** | 1.69 | 1.24 to 2.40 | 0.61 | 0.44 to 1.04 | 0.66 | 0.59 to 0.74 | 0.61 | 0.41 to 0.70 | p = 0.0001 |
| **pep\_275\_280** | 0.53 | 0.46 to 0.60 | 0.18 | 0.01 to 0.21 | 0.19 | 0.10 to 0.20 | 0.09 | 0.01 to 0.21 | p = 0.0001 |
| **pep\_299\_317** | 0.14 | 0.12 to 0.19 | 0.01 | 0.01 to 0.12 | 0.12 | 0.06 to 0.13 | 0.06 | 0.01 to 0.12 | p = 0.0051 |
| **pep\_354\_369** | 0.35 | 0.31 to 0.50 | 0.21 | 0.21 to 0.27 | 0.27 | 0.25 to 0.31 | 0.24 | 0.22 to 0.27 | p = 0.0003 |
| **pep\_386\_395** | 2.16 | 1.04 to 2.95 | 0.57 | 0.01 to 1.24 | 0.26 | 0.01 to 0.59 | 0.68 | 0.53 to 0.80 | p = 0.0008 |
| **pep\_396\_406** | 1.15 | 0.92 to 1.47 | 0.26 | 0.22 to 0.34 | 0.29 | 0.26 to 0.38 | 0.3 | 0.19 to 0.45 | p = 0.0001 |

**Supplementary Table 4.** MS values of the different peptides in AD and non-AD patients (mean and standard deviation, pg/mL of equivalent recombinant tau) in the different clinical groups. Value of Student t-test)

|  |  |  |  |
| --- | --- | --- | --- |
|  | **AD (n=10)** | **Non-AD (n=21)** |  |
|  | **Mean** | **SD** | **Mean** | **SD** | **Student t-test** |
| **pep\_6\_23** | 2.253 | 1.103 | 0.846 | 0.213 | 0.0002 |
| **pep\_25\_44** | 2.248 | 1.834 | 0.570 | 0.555 | <0.0001 |
| **pep\_45\_67** | 1.588 | 0.630 | 0.854 | 0.268 | <0.0001 |
| **pep\_68\_87** | 0.224 | 0.061 | 0.162 | 0.022 | 0.0003 |
| **pep\_88\_126** | 0.165 | 0.038 | 0.113 | 0.034 | <0.0001 |
| **pep\_156\_163** | 15.365 | 6.646 | 5.888 | 1.629 | <0.0001 |
| **pep\_175\_180** | 11.304 | 3.800 | 5.616 | 1.362 | <0.0001 |
| **pep\_181\_190** | 13.470 | 4.644 | 6.080 | 1.508 | <0.0001 |
| **pep\_195\_209** | 16.296 | 8.667 | 4.336 | 1.535 | 0.0001 |
| **pep\_212\_221** | 7.332 | 5.735 | 1.882 | 0.605 | <0.0001 |
| **pep\_212\_224** | 6.512 | 3.220 | 1.895 | 0.548 | <0.0001 |
| **pep\_243\_254** | 1.828 | 1.014 | 0.361 | 0.126 | <0.0001 |
| **pep\_260\_267** | 1.768 | 0.600 | 0.645 | 0.228 | <0.0001 |
| **pep\_275\_280** | 0.503 | 0.158 | 0.119 | 0.104 | <0.0001 |
| **pep\_299\_317** | 0.141 | 0.056 | 0.072 | 0.058 | <0.0001 |
| **pep\_354\_369** | 0.397 | 0.120 | 0.253 | 0.047 | <0.0001 |
| **pep\_386\_395** | 2.363 | 1.433 | 0.621 | 0.438 | <0.0001 |
| **pep\_396\_406** | 1.210 | 0.447 | 0.313 | 0.161 | <0.0001 |