

1 Plasma p-tau₂₁₂ as a biomarker of sporadic and Down Syndrome Alzheimer's 2 disease

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35 **Abstract**

36 **Background**

37 All individuals with Down Syndrome (DS) will develop full-blown Alzheimer’s disease (AD)
38 pathology by age 40, decades before the occurrence of sporadic late-onset AD.
39 Understanding this strong biological relation between age and AD pathology risk in DS is
40 important to accelerate diagnostics, disease monitoring, and treatment. Several genes
41 encoded in chromosome 21 including dual-specificity tyrosine phosphorylation-regulated
42 kinase 1A (DYRK1A) have been proven to contribute to the pathology. A recently validated
43 plasma immunoassay to measure tau phosphorylation at threonine-212 (p-tau212) has very
44 high diagnostic accuracy in detecting AD. P-tau212 is also very sensitive to DYRK1A
45 phosphorylation and is increased in DSAD brain lysates. Here, we assessed the potential of
46 this biomarker in DSAD and sporadic AD.

47 **Methods**

48 Using Simoa technology, we tested p-tau212 and p-tau181 (n=245 for plasma, n=114
49 matching cerebrospinal fluid (CSF) samples). We used AUC-ROC to examine diagnostic
50 performance and the DeLong test to compare the AUC-ROC differences between methods.
51 Spearman correlation is used to examine correlations. Fold changes relative to median levels
52 were calculated for their respective asymptomatic groups. ANCOVA followed by Tukey post-
53 hoc test was used to calculate differences across groups. LOESS was used to determine the
54 temporality of plasma biomarker changes.

55 **Results**

56 We have confirmed that p-tau212 has extremely high accuracy in detecting AD-related
57 changes in euploid controls. For the DS population, we observed a strong correlation
58 between plasma and CSF p-tau212 ($r=0.867$; $p<0.001$). In prodromal DS (pDS) and
59 dementia DS (dDS), we observed significantly elevated levels of p-tau212 in reference to

60 asymptomatic DS (aDS). The diagnostic accuracy to differentiate between aDS and dDS was
61 AUC=0.91 and AUC = 0.86 in discriminating between DS amyloid positive and amyloid
62 negative participants. Plasma p-tau212 started increasing approximately when people
63 became amyloid PET-positive.

64 **Conclusions**

65 We have confirmed that the levels of plasma p-tau212 are increased in the DS population
66 and sporadic AD cases including prodromal and MCI states. Plasma p-tau212 might have
67 utility for theragnostic, monitoring therapy efficacy, and as a target engagement biomarker in
68 clinical trials both in sporadic and DSAD.

69

70 **Keywords**

71 Alzheimer's disease; Down Syndrome; plasma biomarkers; p-tau212; DYRK1A; DABNI;
72 SPIN; Simoa; CSF biomarkers.

73

74 **Introduction**

75 A triplication of chromosome 21 causes Down syndrome (DS)(1). All individuals with DS
76 develop full-blown Alzheimer's Disease (AD) pathology by age 40(2) and the lifetime risk of
77 developing AD exceeds 90% in the seventh decade(3). The estimated age of onset of AD
78 dementia in this population is 53.8 years, decades before the occurrence of late-onset AD in
79 the general population(4). Understanding this strong DSAD relationship is important to
80 accelerate diagnostics and treatment. This ultra-high risk is mainly due to the triplication of
81 the amyloid precursor protein (*APP*), which leads to the overproduction of amyloid- β ($A\beta$)
82 peptides, and increased $A\beta$ plaques formation(5). Although other genes encoded in
83 chromosome 21 such as dual-specificity tyrosine phosphorylation-regulated kinase 1A
84 (*DYRK1A*) and *RCAN1* contribute to the pathology(6–9). *DYRK1A* is a dose-sensitive gene

85 that overexpression contributes to DS cognitive dysfunction(10). This protein phosphorylates
86 different targets involved in AD development and progression for instance Glycogen
87 synthase kinase-3 β (GSK-3 β)(11), Presenilin1(12), APP(6), and tau(13).

88

89 Diagnosing DSAD is challenging due to the association of DS with cognitive
90 dysfunction(14,15). Recent advances allow us to recognize ante-mortem AD pathology using
91 blood-based biomarkers(16–19). These reflect the disease almost as accurately as more
92 expensive and less available CSF and imaging biomarkers(20). Several plasma p-tau
93 biomarkers have been found to reflect both A β and tau pathology(21). Recently validated
94 plasma p-tau₂₁₂ has shown very high performance for detecting those pathologies and AD
95 diagnosis(22). That phosphorylation site might also have the strongest biological correlation
96 with AD pathology in DS since threonine-212 is a primary target for DYRK1A in tau
97 protein(23). Intensified phosphorylation at thr-212 induces tau aggregation, reduces tau
98 binding to microtubules, and increases cell toxicity in in-vitro studies(24). Levels of p-tau₂₁₂
99 are highly elevated in reference to AD and control participants in human AD-DS brains(25).
100 Additionally, a major tau phosphatase – Protein phosphatase 1A (PP1A) is not
101 dephosphorylating p-tau₂₁₂ derived from AD brains(26), suggesting that this epitope might
102 be vulnerable to very subtle changes related to AD pathology. Knowing that direct biological
103 association, we hypothesized that p-tau₂₁₂ is an accurate tau species to reflect AD
104 pathology in the DS population. To test this hypothesis, we used Single molecule array
105 (Simoa) immunoassays to measure plasma and cerebrospinal fluid (CSF) p-tau₂₁₂
106 concentrations in asymptomatic DS (aDS), prodromal DS (pDS), and dementia DS (dDS)
107 individuals, as well as in sporadic AD patients both in Mild Cognitive impairment (MCI) and
108 dementia states and we compare the results with a validated biomarker.

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110

111

112

113 **Methods**

114 **Study design and participants**

115 We performed a cross-sectional cohort study of adults with DS, and euploid individuals along
116 the Alzheimer's disease continuum in the Hospital of Sant Pau, Barcelona (Spain), Adults
117 with DS in Barcelona were recruited from a population-based health plan designed to screen
118 for AD dementia, which includes yearly neurological and neuropsychological assessments.
119 Those subjects interested in research studies are included in the Down Alzheimer Barcelona
120 Neuroimaging Initiative (DABNI) cohort(14,15). We also recruited euploid controls and
121 sporadic Alzheimer's disease patients from the Sant Pau Initiative on Neurodegeneration
122 (SPIN cohort)(27).

123

124 **Biomarker measurements**

125 Amyloid- β peptides (A β 40, A β 42) analyses were performed on the LUMIPULSE G600II, and
126 the cut-off for positivity was defined as in the previous publication(28). All other plasma and
127 CSF biomarker assays were performed on the Simoa HD-X platform at the University of
128 Gothenburg. P-tau212 and p-tau181 concentrations were measured using published and
129 validated in-house assays(16,22). For p-tau181, the AT270 antibody (Invitrogen) was used
130 as a capture antibody and was paired with n-terminal antibody for detection (Tau12;
131 BioLegend). For p-tau212, a sheep monoclonal antibody was used for capture paired with
132 Tau12 as the detector. Coefficients of variation for 3 different internal quality controls for
133 plasma were 5.3-12% for within-plate variation and 6.4%-12% for between-plate variation.
134 For CSF, these values were 10.8%-13.3% and 12.9%-15.5%, respectively.

135

136 **Statistical Analysis:**

137 We used AUC-ROC to examine diagnostic performance and the DeLong test to compare the
138 AUC-ROC differences between methods. Spearman correlation is used to examine

139 correlations. Fold changes relative to median levels are calculated for their respective
140 asymptomatic groups. Age-adjusted analysis of covariance (ANCOVA) followed by Tukey
141 post-hoc test is used to calculate differences across groups. A First-degree locally estimated
142 scatterplot smoothing curve (LOESS) is independently used in controls and adults with Down
143 Syndrome to determine the temporality of plasma biomarker changes. For Down participants
144 we set the mean age of symptoms onset at 53.8 years according to a previous study from
145 our group(29). All significance tests were two-sided, and significance was set at $p<0.05$.

146

147 **Results**

148 **Cohort characteristics**

149 We tested $n=245$ plasma samples for p-tau212 and p-tau181. A subset of participants had
150 amyloid PET data. Table 1. shows the demographics, cognitive, and plasma biomarkers
151 across groups of all participants included in the analyses. $N=114$ (47%) participants had CSF
152 biomarker measurements. Demographics for the CSF subset are shown in Supplementary
153 Table 1.

154

155 **Correlations with biomarkers and cognition**

156 Plasma and CSF p-tau212 were highly correlated with each other within the cohort. We
157 observed moderate correlation across sample types in all participants ($r=0.712$ $p<0.0001$)
158 (Fig. 1A). However, the strongest correlation of plasma p-tau212 with CSF p-tau212 was
159 observed in the DS-only subgroup ($r=0.867$, $p<0.0001$) (Fig 1A). This improved CSF-plasma
160 correlation in DS groups was not seen for p-tau181, which showed similar correlations in the
161 whole cohort and subgroups ($r=0.652-0.681$, $p<0.0001$) (Fig 1B). Those results indicate that
162 plasma p-tau212 measurements accurately reflect AD-related p-tau level changes in CSF in
163 sporadic and DS groups.

164

165 **Figure 1. Spearman correlations for plasma and CSF p-tau212 and p-tau181.** The Figure
166 shows correlations between plasma and CSF for **A)** p-tau212 and **B)** p-tau181 for euploid
167 groups (n=88; p<0.001; r= 0.701 and r=0.660 respectively) and DS groups (n=26, p<0.001;
168 r=0.867, r=0,681 respectively). The correlation between plasma and CSF measurements for
169 all measurements (n=114, p<0.001) is 0.712 for p-tau212 and 0.652 for p-tau181. The fitted
170 simple linear regression line is presented as a mean and error.

171
172 Both biomarkers were correlated with decrease in The Cambridge Cognitive Examination
173 adapted for individuals with Down Syndrome (CAMCOG-DS) in plasma (r=-0.338; p=0.001
174 for p-tau212 and r=-0.328 for p-tau181; p=0.002;) and in CSF (r=-0.686; p<0.001 for p-
175 tau212; and r=-0.511; p<0.001 for p-tau181).

176

177 **Plasma p-tau212 levels are increased in asymptomatic Down syndrome**

178 Plasma p-tau212 concentration was 2.4x times higher in the aDS group compared with
179 cognitively normal (CN) euploid people (p<0.001) whereas plasma p-tau181 was not
180 significantly changed (p=0.052).

181

182 **Plasma and CSF p-tau212 increase along the AD continuum in DSAD and sporadic AD**

183 Both in individuals with DS and euploid participants, p-tau212 levels were increased in
184 symptomatic patients in comparison with asymptomatic individuals. (Fig. 2A). For pDS we
185 observed a 3.4x (p=0.003) mean fold increase in plasma and a 5.6x mean fold-fold increase
186 in CSF in reference to aDS. For dDS we observed a 2.9x (p=0.004) mean fold increase in
187 plasma and a 7.1x mean fold increase in CSF. For MCI-AD, compared with cognitively
188 normal euploid people, we observed a 3.0x (p<0.001) mean fold increase in plasma and a
189 7.8x mean fold increase in CSF. AD dementia patients had a 3.8x mean fold increase in
190 plasma (p<0.001) and a 9.1x mean fold increase in CSF. P-tau181 concentrations were also
191 increased, but with a lower magnitude than p-tau212, and comparison between DS groups
192 showed no significance (Fig. 2B). Both biomarkers kept the pattern of greater increases in
193 CSF than in plasma (Supplementary Fig. 1A-B).

194

195 **Figure 2. Plasma p-tau212 and p-tau181 levels in euploid and Down Syndrome (DS)**
196 **groups.** Box plots represent median and IQR, and boundaries of the whiskers are minimum
197 to maximum values for **A)** plasma p-tau212 and **B)** plasma p-tau181. Differences for euploid

198 participants are calculated for Mild Cognitively impaired Alzheimer's Disease (MCI-AD; n=62)
199 and Alzheimer's Disease Dementia (AD; n=20) in reference to Cognitively Normal (CN;
200 n=46) participants. Differences for prodromal Alzheimer's Disease in DS (pDS; n=8) and
201 Alzheimer's Disease dementia in DS (dDS; n=17) are calculated in reference to
202 asymptomatic (aDS; n=92). Age-adjusted analysis of covariance (ANCOVA) followed by
203 Tukey post-hoc test is used to calculate differences across groups.
204

205 **Plasma p-tau212 has greater diagnostic accuracy than p-tau181**

206 ROC analysis was used to evaluate the diagnostic performance of plasma and CSF p-tau212
207 and p-tau181. AUCs were usually higher for biomarkers in CSF, and for p-tau212 than for p-
208 tau181. In our comparisons, we included Age+ Apolipoprotein E4 (APOE4)+Sex since they
209 have been shown to influence diagnostic accuracy(30). AUCs of Age+APOE+Sex were not
210 significantly different from p-tau212 but better than p-tau181. Both biomarkers had high
211 accuracy to differentiate between CN and AD in plasma AUC = 0.96 (95% CI 0.92-1) for p-
212 tau212 and AUC = 0.89 (95% CI 0.84-0.95) for p-tau181 (Fig. 3B). For differentiating
213 between CN and MCI-AD (Fig. 3A) or MCI-AD+AD (Fig. 3C), p-tau212 had accuracy of 0.91
214 (95% CI 0.85-0.97) and AUC=0.93 [95% CI 0.88-0.97] respectively. That accuracy was
215 significantly higher than p-tau181 - (p=0.026 for both comparisons). Plasma p-tau212
216 reached AUC=0.91 (95% CI 0.86-0.97) to differentiate between aDS and dDS diagnosis (Fig.
217 3D).

218

219 **Figure 3. Diagnostic accuracy of plasma and CSF biomarkers to discriminate between**
220 **sporadic and DSAD groups.** P-tau212 and p-tau181 receiver operating characteristic
221 curves (ROC) to discriminate between sporadic and DSAD groups. Plasma p-tau181, plasma
222 p-tau212, CSF p-tau181, CSF p-tau212, and Age+APOE+Sex are on each graph. In **A**) ROC
223 curves for differentiating Cognitively normal (CN) and Mild Cognitive Impairment -
224 Alzheimer's Dementia (MCI-AD). **B**) ROC curves to discriminate between CN and
225 Alzheimer's Disease (AD). **C**) ROC curves to differentiate between CN and MCI-AD
226 combined with AD group. **D**) ROC curves to discriminate between asymptomatic Down
227 syndrome (aDS) and prodromal Down Syndrome (pDS) + dementia Down syndrome (dDS).
228

229 **Plasma p-tau212 increases approximately parallel to amyloid PET positivity and has**
230 **great accuracy in discriminating between A β + and A β - participants**

231 Both biomarkers had significant accuracy in discriminating between A β + and A β - participants
232 (Fig. 4). Plasma p-tau212 had numerically higher accuracy than p-tau181 both for DS (AUC
233 = 0.86 (95%CI = 0.7-1) (Fig. 4B) and euploid participants AUC = 0.9 (0.83 – 0.96) (Fig. 4C).

234
235 **Figure 4. Diagnostic accuracy of plasma and CSF biomarkers to discriminate between**
236 **A β + and A β - participants.** P-tau212 and p-tau181 receiver operating characteristic curves
237 (ROC) to discriminate between amyloid positive (A β +) and amyloid negative (A β -)
238 participants in sporadic and DSAD groups. Plasma p-tau181, plasma p-tau212, CSF p-
239 tau181, CSF p-tau212, and Age+APOE+Sex are on each graph. In **A**) ROC curves for
240 differentiating A β + from A β - in whole cohort. **B**) ROC curves to discriminate A β + and A β - in
241 DS groups. **C**) ROC curves to differentiate between A β + and A β - in euploid groups.
242

243 An early increase in plasma levels was observed many years before the onset of clinical AD
244 symptoms in DS (Fig 5). For p-tau212 the increase started approximately when people
245 became amyloid PET-positive, *i.e.*, in their late 30s, and approximately 15 years before the
246 disease onset (Fig. 5A). P-tau181 started increasing approximately 10 years before the
247 estimated disease onset (Fig. 5B).

248
249 **Figure 5. Age-related plasma p-tau212 and p-tau181 changes in Down syndrome and**
250 **euploid controls.** Open Circles represent asymptomatic and filled circles represent
251 symptomatic participants. Down syndrome population is represented in red and cognitively
252 normal euploid people are represented in blue. Horizontal lines depict each group's fitted
253 loess model, and faded bands display confidence intervals. The vertical red line represents
254 the estimated years to symptom onset which is 53.8 for Down syndrome participants and
255 was used as reference in euploid controls for comparison purposes.
256

257 Discussion

258 In this cross-sectional study, we show that p-tau212 serves as a biomarker to track AD-
259 related changes in sporadic cases and DSAD. P-tau212 has a very high correlation between
260 plasma and CSF. The strongest correlation was observed for the DS population, where p-
261 tau212 reached a greater correlation than for euploid participants. For p-tau181 correlation in
262 euploid and DS groups was similar, suggesting greater translation from CSF to plasma for p-
263 tau212 in DS populations.

264

265 We observed elevated p-tau212 concentration in plasma and CSF in aDS compared with
266 euploid CN. That elevation is greater than the p-tau181 elevation, which did not reach
267 statistical significance. Additionally, other reported biomarkers, such as p-tau217 Glial
268 fibrillary acidic protein (GFAP)(30,31), had lower increases. This suggests the effects of
269 DYRK1A gene dose interacting with AD pathology on p-tau212 levels in DS and a better fit
270 for the use of this biomarker in the DS population.

271

272 Levels of plasma p-tau212 were significantly higher across disease groups. Plasma p-tau212
273 reached significance levels to differentiate between aDS vs pDS and aDS vs dDS groups
274 while p-tau181 failed to do it. However, we think this might be a limitation of the small sample
275 size used in this cohort since p-tau181 levels were previously shown to be significantly
276 increased in pDS and aDS(30,32). In MCI-AD and AD dementia groups, we observed
277 significant increases for both biomarkers, concomitantly having fold changes higher for p-
278 tau212 which confirms our previous findings(22).

279

280 The excellent performance of the assay was confirmed in discriminating patients according to
281 the diagnosis in both DS and euploid groups. P-tau212 additionally has greater accuracy
282 than p-tau181 in discriminating between MCI-AD and control groups, simultaneously
283 reaching 0.96 AUC to differentiate CN from AD. Additionally, plasma p-tau212 acquired very
284 high AUC-ROC in discriminating between A β + and A β - participants in both euploid and DS
285 groups. This accuracy was not different from CSF accuracy, supporting the high between-
286 matrix translation of p-tau212 and providing additional reasoning to use plasma p-tau212 to
287 recruit participants for clinical trials.

288

289 Plasma p-tau212 starts increasing in the 30s, approximately when people start being positive
290 in amyloid PET scans, and 5 years before we observe an increase in p-tau181. Additionally,
291 the biomarker increased further as AD progressed towards symptomatic stages. Therefore p-
292 tau212 could be useful to monitor the progression of asymptomatic DS people to prodromal

293 AD. Moreover, the onset of the increase comes along with the appearance of neurofibrillary
294 tangles (NFTs) and A β plaques in brain(4,33).

295

296 This is (to our best knowledge) the first study to measure p-tau212 in CSF and plasma as a
297 biomarker for DSAD however, the potential involvement of p-tau212 in this population and its
298 association with DYRK1A was published more than 20 years ago(13). The kinase is
299 perceived as a target in DS and neurodegenerative diseases(8,34). Its under- or
300 overexpression leads to different clinical phenotypes including cognitive impairment(34).
301 Since DYRK1A is a dose-sensitive protein in which down-regulation or up-regulation has a
302 critical role, DYRK1A inhibitors have already been widely explored in clinical trials and have
303 been proven to improve cognitive function in DS people(10,35,36). Importantly, p-tau212 has
304 already been successfully used to test the efficacy of DYRK1A inhibitor in cell models(37).
305 The use of the chosen inhibitor was further shown to reverse the upregulation of p-tau212 in
306 hippocampal tissue and temporal cortex in mouse models(37). Our novel plasma p-tau212
307 immunoassay provides a simple-to-implement and cost-effective opportunity to monitor the
308 efficacy of DYRK1A inhibitors, or in the future – enhancers, not allowing the activity of this
309 kinase to be reduced or increased to levels that could cause more harm than good. This
310 utility will be explored in our future research.

311

312 The major strength of this study is the confirmation that p-tau212 is increased in the DS
313 population, and levels of this biomarker increase with progression to AD dementia. P-tau212
314 reaches very high accuracy to differentiate between control and disease groups and A β + and
315 A β - participants. The high correlation between plasma and CSF p-tau212 also supports a
316 very high translation of the results from CSF to plasma. DeLong tests between DS groups
317 did not show any significantly better performance of CSF p-tau212 compared with plasma p-
318 tau212 providing further evidence that plasma measurements can be used for clinical
319 evaluation of AD pathophysiological processes occurring in patients with suspected disease.
320 Advantages would also be reflected in the economy, availability, and perception of the test,

321 since lumbar punctures or PET scans are costly, require resources, and might be perceived
322 as frightening(20). Next, plasma p-tau212 increased approximately when people are starting
323 to be amyloid PET positive and 5 years before p-tau181, indicating the benefits in disease
324 monitoring.

325

326 This study has a few limitations. First is a slightly low representation of prodromal-DS
327 participants, which prohibits us from making better AUC-ROC analysis in that group. Ideally,
328 longitudinal measurements of p-tau212 in the DS population would tell us more about the
329 trajectories of this biomarker. The second limitation is that p-tau217 measurements are
330 unavailable at the moment, however direct comparison between biomarkers was not a
331 purpose of experiments presented in this article. Still, p-tau181 is the most commercialized
332 and fully automated immunoassay, with great utility in AD.

333

334 **Conclusions**

335 In conclusion, we have confirmed that levels of plasma p-tau212 are increased in the DS
336 population and sporadic AD cases including prodromal and MCI states. High accuracy in
337 discriminating amyloid positive from amyloid negative people and increase in parallel to
338 amyloid-PET positivity give the promise to evaluate ongoing pathophysiological AD
339 processes many years before the disease onset in individuals with DS. This will also facilitate
340 participants recruitment for clinical trials. This is a cost-effective application that provides
341 higher chance to receive appropriate therapy. Plasma p-tau212 will also find high utility for
342 theragnostic, to monitor therapy efficacy, and as a target engagement biomarker in clinical
343 trials both in sporadic and DSAD.

344

345 **Abbreviations**

346 AD: Alzheimer's disease

- 347 DS: Down Syndrome
- 348 DYRK1A: dual-specificity tyrosine phosphorylation-regulated kinase 1A
- 349 p-tauX: tau phosphorylated at amino acid X
- 350 DSAD Down Syndrome Alzheimer's disease
- 351 CSF Cerebrospinal Fluid
- 352 AUC-ROC: Area Under the Curve and Receiver Operating Curves
- 353 ANCOVA: Age-adjusted analysis of covariance
- 354 LOESS: locally estimated scatterplot smoothing
- 355 pDS: prodromal Down syndrome
- 356 aDS: asymptomatic Down syndrome
- 357 dDS: dementia Down syndrome
- 358 PET: Positron Emission Tomography
- 359 MCI: mild cognitive impairment
- 360 A β : amyloid beta
- 361 APP: amyloid precursor protein
- 362 GSK-3 β : Glycogen synthase kinase-3 β
- 363 PP1A: Protein phosphatase 1A
- 364 DABNI: Down Alzheimer Barcelona Neuroimaging Initiative
- 365 SPIN: Sant Pau Initiative on Neurodegeneration

366 CN: cognitively normal

367 IQR; interquartile range

368 SD: standard deviation

369 CAMCOG-DS: The Cambridge Cognitive Examination adapted for individuals with Down
370 Syndrome

371 APOE4: Apolipoprotein E4

372 GFAP: Glial fibrillary acidic protein

373 NFTs: Neurofibrillary tangles

374

375 **Declarations**

376 **Ethics approval and consent to participate**

377 Study procedures were approved by the Sant Pau Ethics Committee (IIBSP-NGF-2018-36
378 and IIBSP-DOW-2014-30), following the standards for medical research in humans, as
379 recommended in the Declaration of Helsinki. All participants or their legally authorized
380 representative gave written informed consent before enrolment.

381

382 **Consent for publication**

383 Not applicable

384

385 **Availability of data and materials**

386 Blinded Anonymized data is available on reasonable request from the corresponding author.
387 Request will be reviewed by the investigators and respective institutions to verify if data
388 transfer is in the agreement with EU legislation on the general data protection or is subject to
389 any intellectual property or confidentiality obligations.

390

391 **Competing interests**

392 MT and PH are employees of Bioventix Plc. HZ has served at scientific advisory boards
393 and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon,
394 Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai,
395 LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon
396 Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens
397 Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by
398 Alzecure, Biogen, Celectricon, Fujirebio, Lilly, Novo Nordisk, and Roche, and is a co-founder
399 of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures
400 Incubator Program (outside submitted work). KB has served as a consultant or at advisory
401 boards for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu,
402 Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens
403 Healthineers. HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a
404 GU Ventures-based platform company at the University of Gothenburg. D.A. participated in
405 advisory boards from Fujirebio-Europe, Roche Diagnostics, Grifols S.A. and Lilly, and
406 received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Krka
407 Farmacéutica S.L., Zambon S.A.U. and Esteve Pharmaceuticals S.A. D.A. and JF declare a
408 filed patent application (licensed to Adx, EPI8382175 J.F. reported receiving personal fees
409 for service on the advisory boards, adjudication committees or speaker honoraria from AC
410 Immune, Adamed, Alzheon, Biogen, Eisai, Esteve, Eisai, Fujirebio, Ionis, Laboratorios
411 Carnot, Lilly, Life Molecular Imaging, Lundbeck, Perha, Roche, and, outside the submitted
412 work. The other authors declare no competing interest.

413

414 **Funding**

415 PRK was funded by Demensförbundet and Anna Lisa and Brother Björnsson's Foundation.
416 LMG is supported by the Brightfocus Foundation (A2022015F), the Swedish Dementia

417 Foundation, Gun and Bertil Stohnes Foundation, Åhlén-stifelsen, Alzheimerfonden (AF-
418 968621) and Gamla Tjänarinnor Foundation. FG-O was funded by the Anna Lisa and Brother
419 Björnsson's Foundation. HZ is a Wallenberg Scholar and a Distinguished Professor at the
420 Swedish Research Council supported by grants from the Swedish Research Council (#2023-
421 00356; #2022-01018 and #2019-02397), the European Union's Horizon Europe research and
422 innovation programme under grant agreement No 101053962, Swedish State Support for
423 Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF),
424 USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-
425 21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C, and #ADSF-24-1284328-C), the
426 Bluefield Project, Cure Alzheimer's Fund, the Olav Thon Foundation, the Erling-Persson
427 Family Foundation, Familjen Rönströms Stiftelse, Stiftelsen för Gamla Tjänarinnor,
428 Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and
429 innovation programme under the Marie Skłodowska-Curie grant agreement No 860197
430 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research
431 (JPND2021-00694), the National Institute for Health and Care Research University College
432 London Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at
433 UCL (UKDRI-1003). KB is supported by the Swedish Research Council (#2017-00915 and
434 #2022-00732), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721, #AF-968270,
435 and #AF-994551), Hjärnfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish
436 state under the agreement between the Swedish government and the County Councils, the
437 ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), the European Union Joint
438 Program for Neurodegenerative Disorders (JPND2019-466-236), the Alzheimer's Association
439 2021 Zenith Award (ZEN-21-848495), the Alzheimer's Association 2022-2025 Grant (SG-23-
440 1038904 QC), La Fondation Recherche Alzheimer (FRA), Paris, France, the Kirsten and
441 Freddy Johansen Foundation, Copenhagen, Denmark, and Familjen Rönströms Stiftelse,
442 Stockholm, Sweden. TKK was supported by the NIH (R01 AG083874, RF1 AG052525-01,
443 P30 AG066468-04, R01 AG053952, R01 MH121619, R37 AG023651, RF1 AG025516-12A1,
444 R01 AG073267, R01 MH108509, R01 AG075336, R01 AG072641, P01 AG025204), the

445 Swedish Research Council (Vetenskåpradet; #2021-03244), the Alzheimer's Association
446 (#AARF-21-850325), the Swedish Alzheimer Foundation (Alzheimerfonden), the Aina (Ann)
447 Wallströms and Mary-Ann Sjöbloms stiftelsen, and the Emil och Wera Cornells stiftelsen. J.F
448 was supported by the Fondo de Investigaciones Sanitario, Carlos III Health Institute
449 (INT21/00073, PI20/01473 and PI23/01786) and the Centro de Investigación Biomédica en
450 Red sobre Enfermedades Neurodegenerativas Program 1, partly jointly funded by Fondo
451 Europeo de Desarrollo Regional, Unión Europea, Una Manera de Hacer Europa. This work
452 was also supported by the National Institutes of Health grants (R01 AG056850; R21
453 AG056974, R01 AG061566, R01 AG081394 and R61AG066543), the Department de Salut
454 de la Generalitat de Catalunya, Pla Estratègic de Recerca i Innovació en Salut
455 (SLT006/17/00119). It was also supported by Fundació Tatiana Pérez de Guzmán el Bueno
456 (IIBSP-DOW-2020-151) and Horizon 2020–Research and Innovation Framework Programme
457 from the European Union (H2020-SC1-BHC-2018-2020).

458

459 **Authors' contributions**

460 PRK, DA, NJA, HZ, TKK, JF and KB created the concept and design. Data acquisition and
461 analysis was performed by PRK, DA, LMG, JLR, NJA, JF. SF, LM, BB, MT, IB, PH, LV, AL,
462 MC-I contributed to the sample selection/and or interpretation of the data. PKK, DA, LMG,
463 JLR, FG-O, HZ, TKK, JF and KB drafted the manuscript, and all authors revised. All authors
464 read and approved the final manuscript.

465

466 **Acknowledgements**

467 We thank all the participants with Down syndrome, their families, and their carers for their
468 support of, and dedication to this research. We also acknowledge Fundació Catalana
469 Síndrome de Down for global support and the members of the Alzheimer Down Unit and the
470 Memory Unit from Hospital de la Santa Creu i Sant Pau for their daily work and dedication.

471

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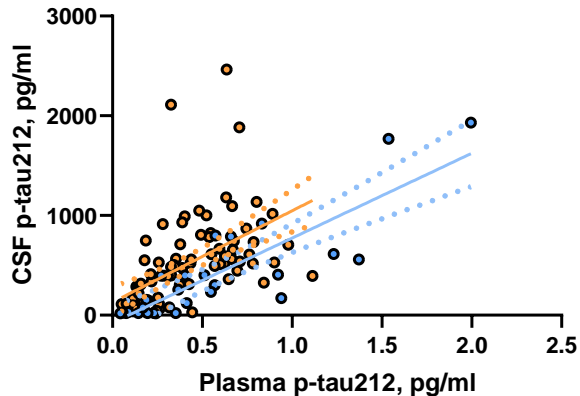
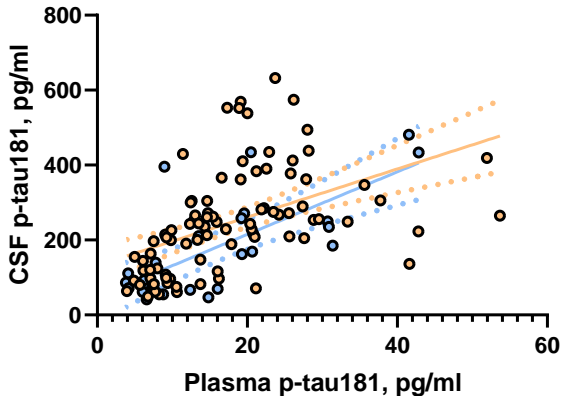
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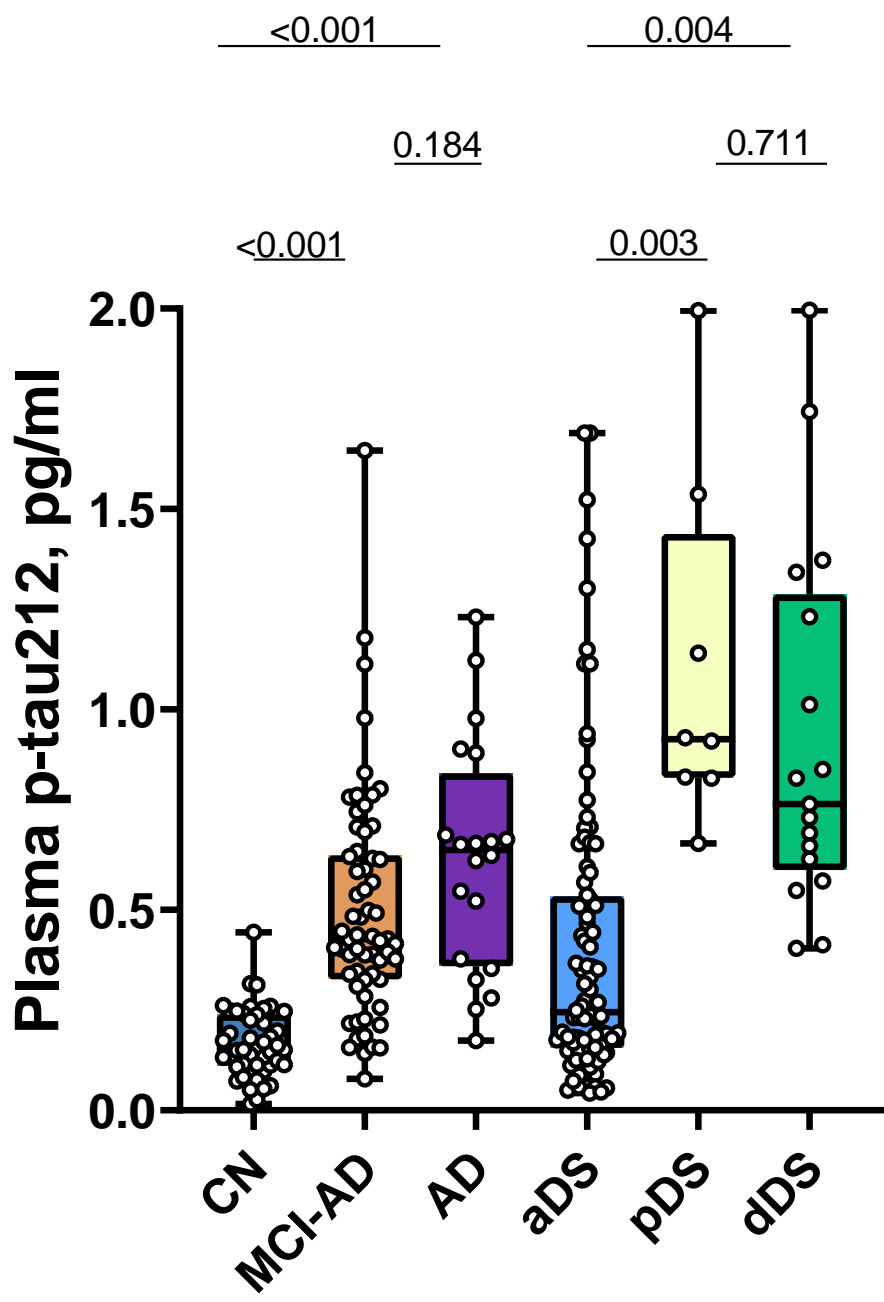
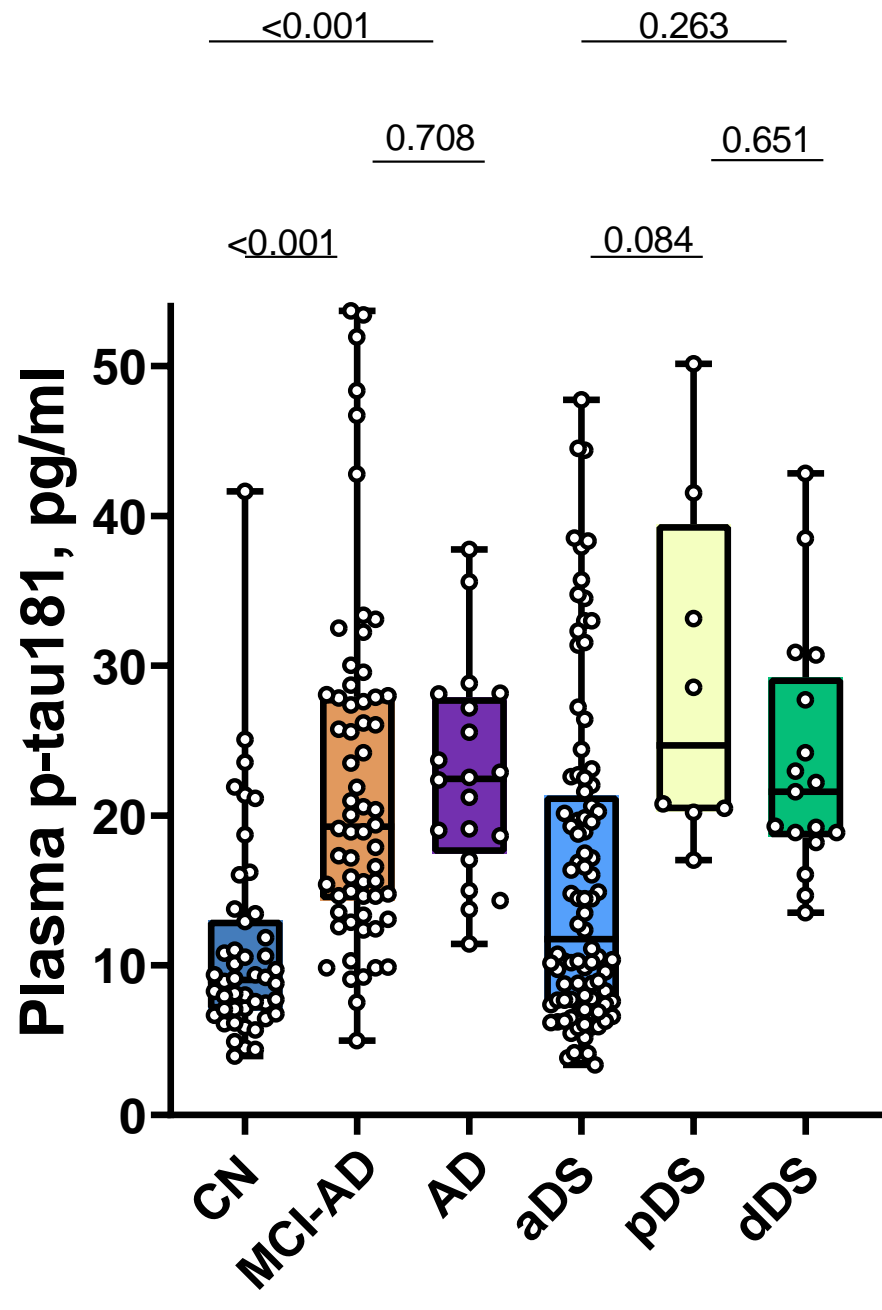
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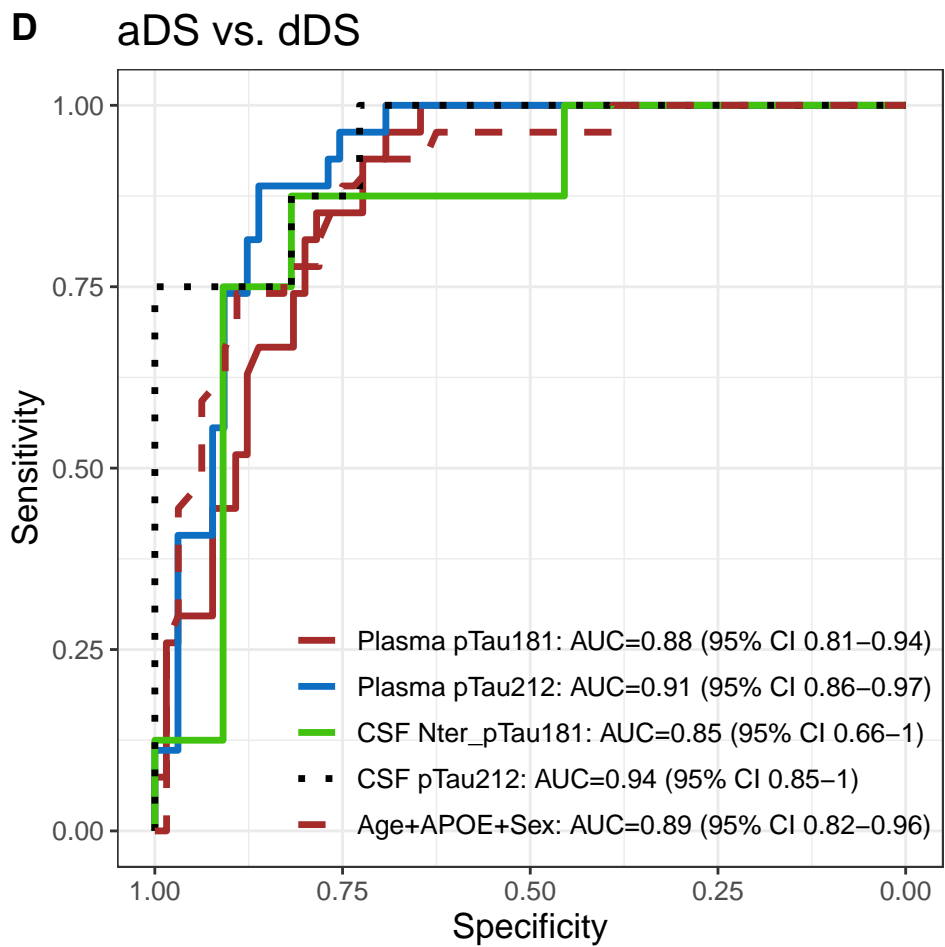
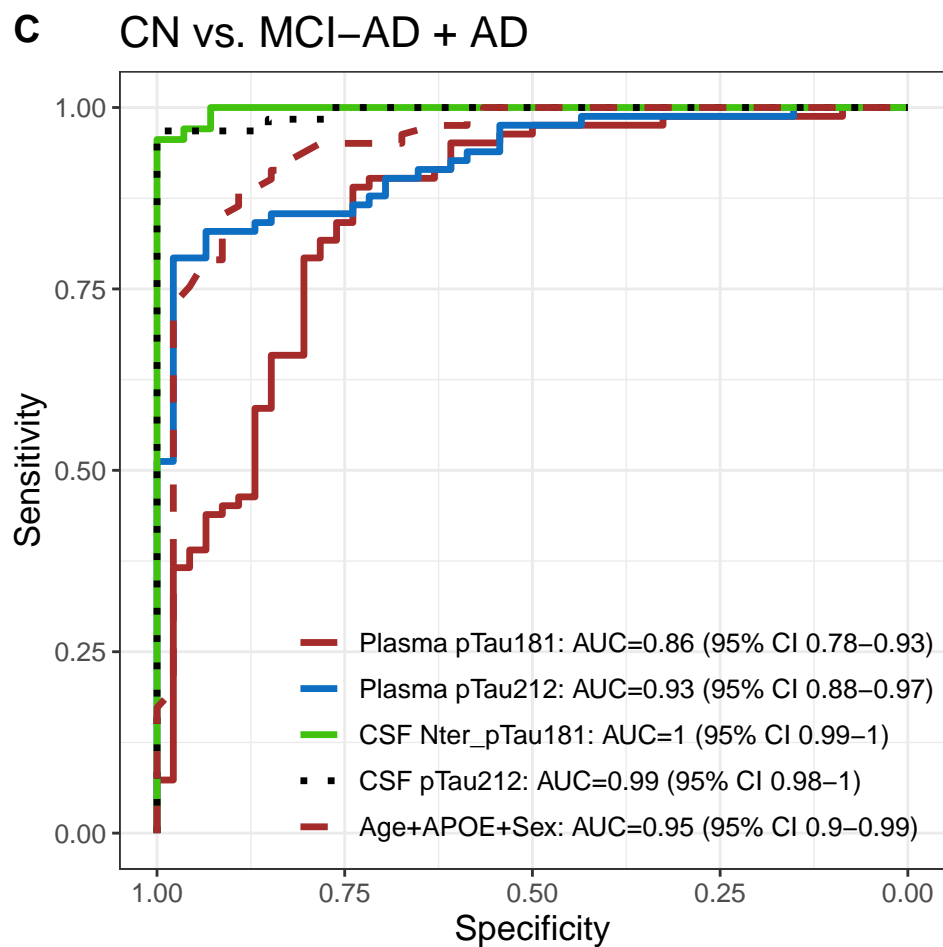
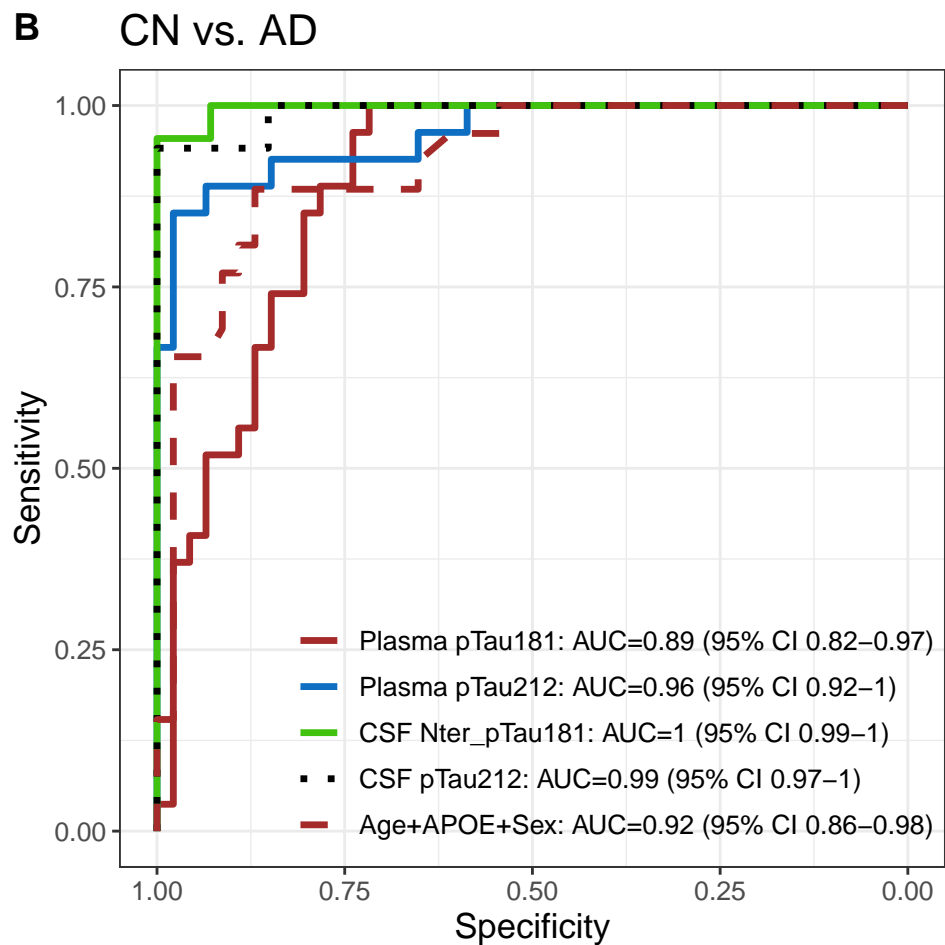
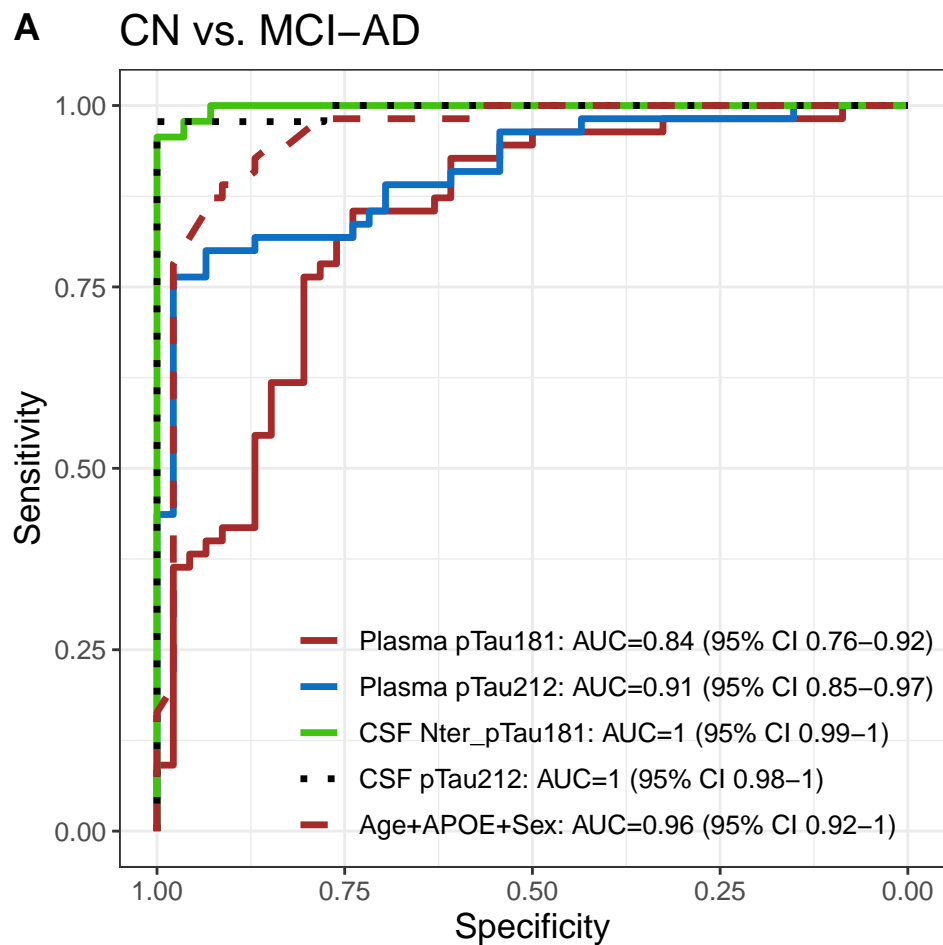
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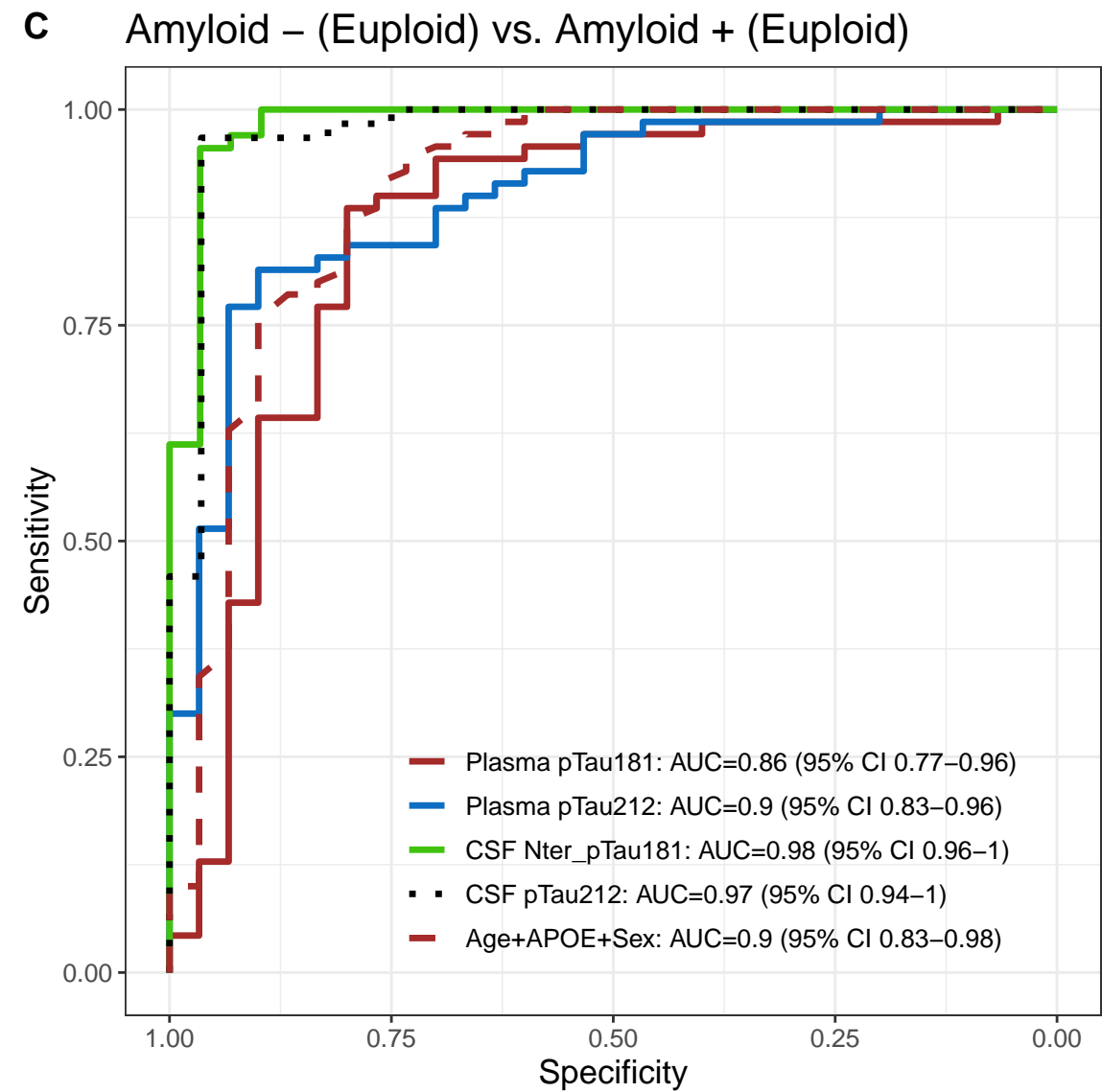
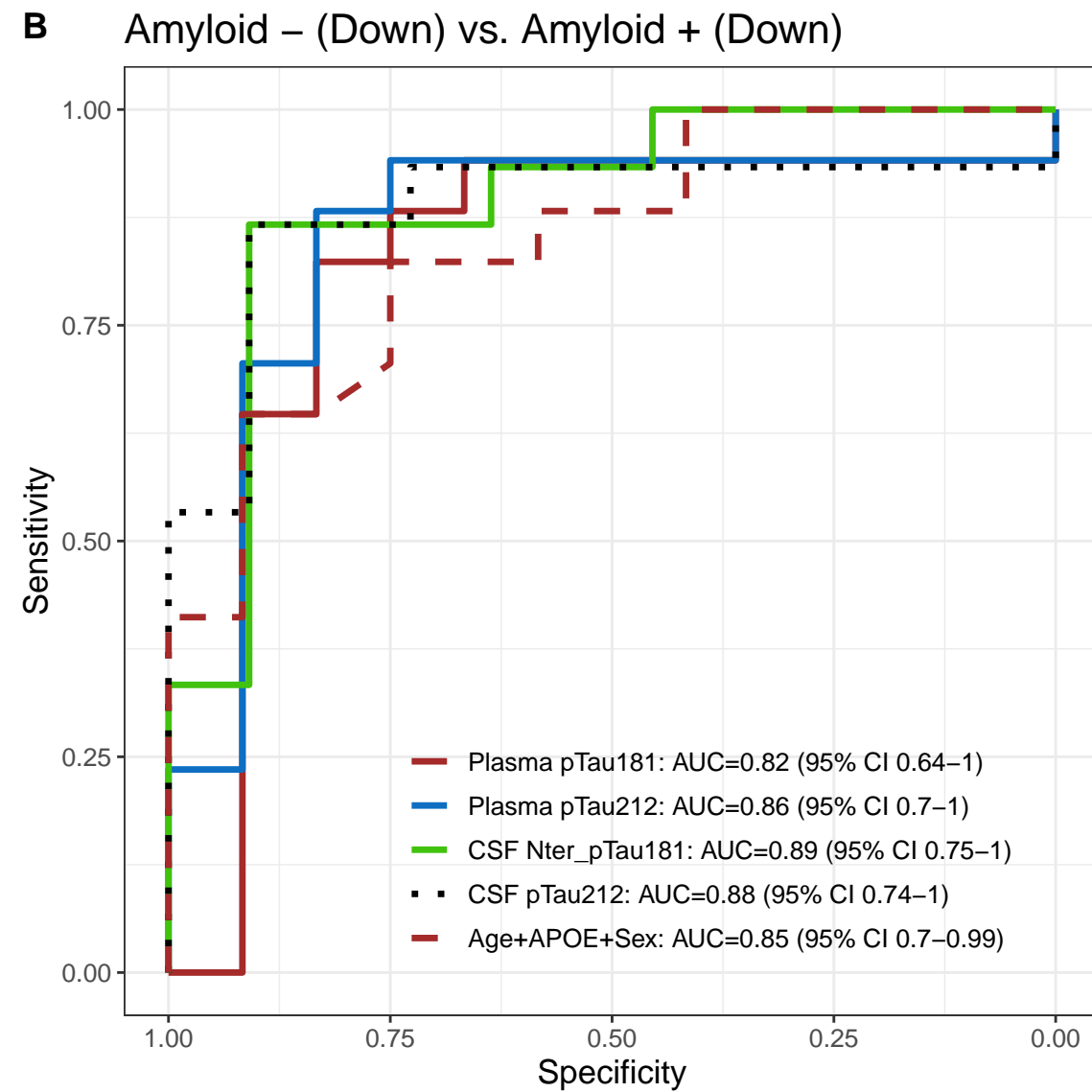
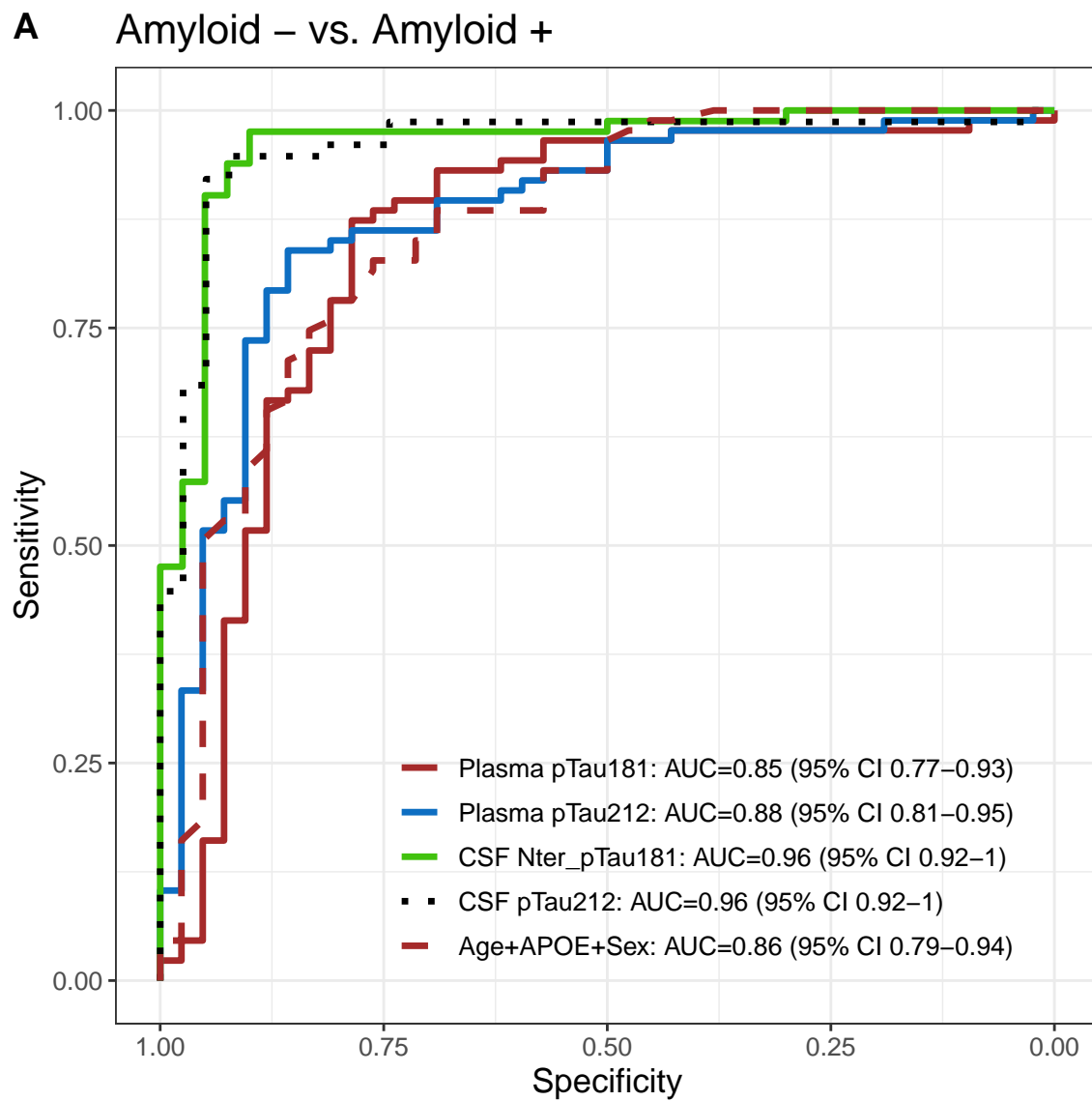
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A**P-tau212 Spearman correlation** $r=0.7012$ (95% CI 0.5722 - 0.7963) ● Euploid groups $r=0.8674$ (95% CI 0.7170 - 0.9406) ● DS groups**B****P-tau181 Spearman Correlation** $r=0.6602$ (95% CI 0.5185 - 0.7666) ● Euploid groups $r=0.6807$ (95% CI 0.3882 - 0.8486) ● DS groups

A**B**

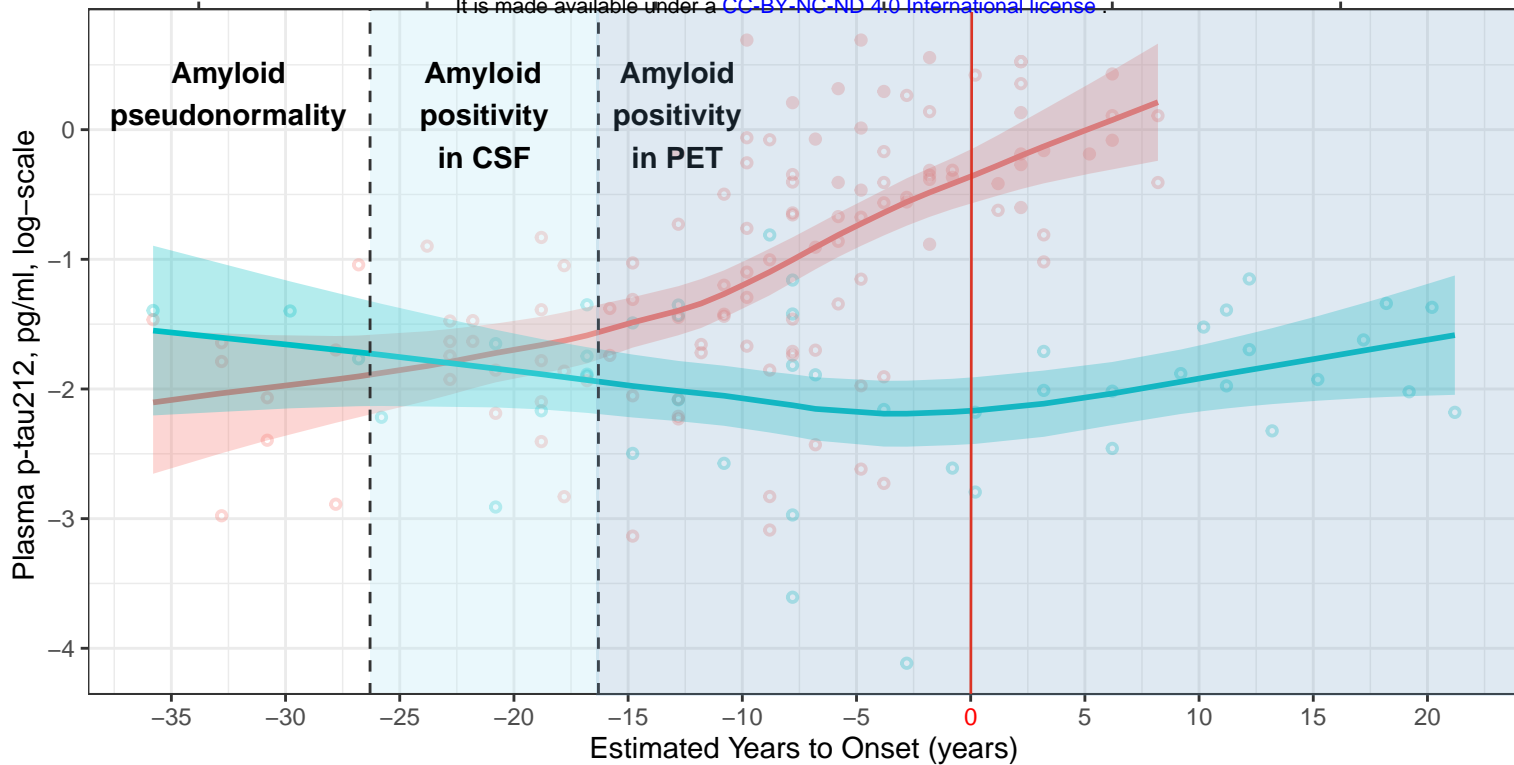




A

○ Asymptomatic ● Symptomatic ● Down ● Euploid CN

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**B**

○ Asymptomatic ● Symptomatic ● Down ● Euploid CN

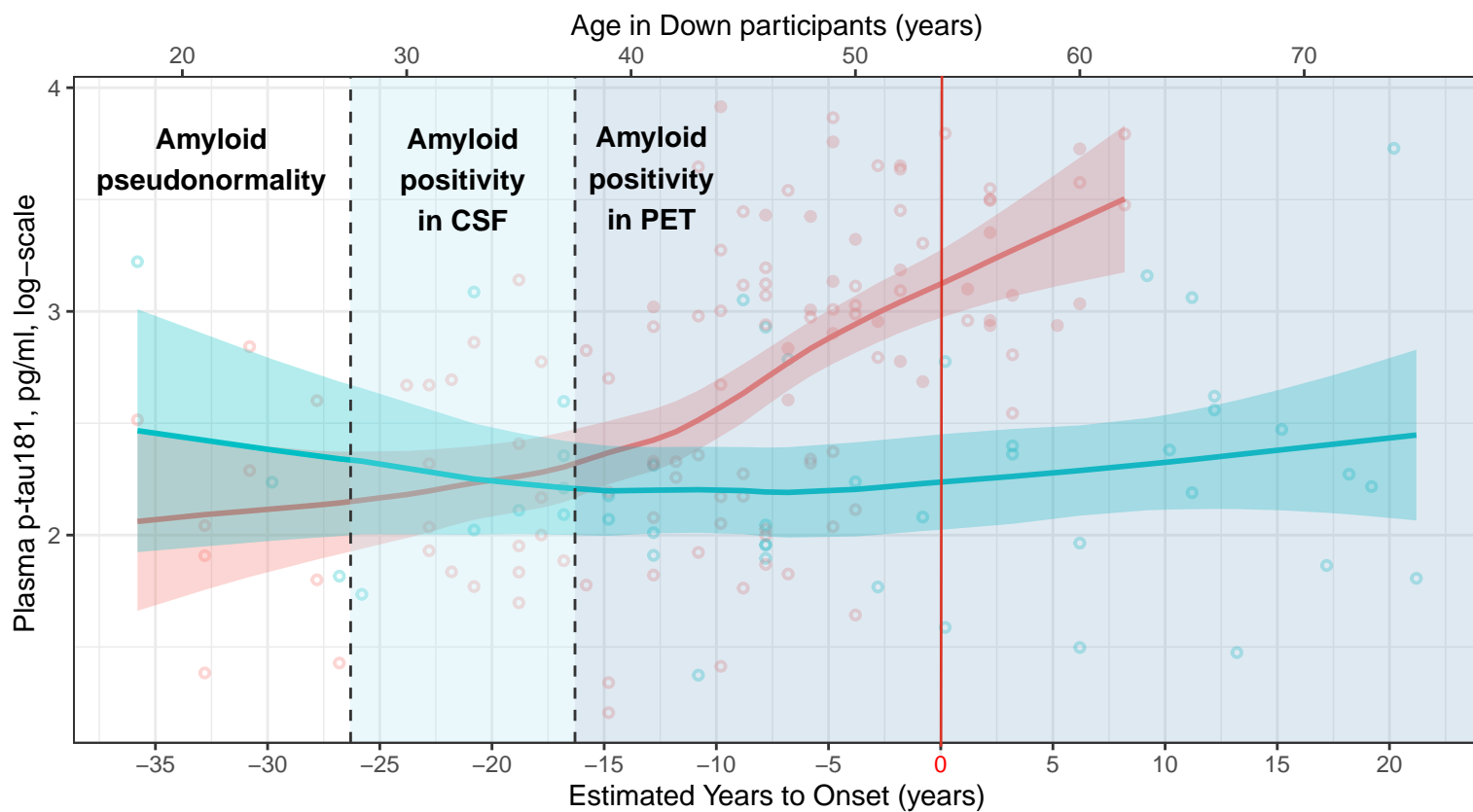


Table 1. Study Participants

	aDS	pDS	dDS	CN	MCI-AD	AD
n=	36/56	5/3	8/9	31/15	35/27	12/8
Female/Male	(39.1/60.9%)	(62.5/37.5%)	(47.1/52.9%)	(67.4/32.6%)	(56.5/43.5%)	(60/40%)
AGE (years)-range	18-62	41-60	46-59	18-75	56-83	53-83
AGE (years)-mean(sd)	42.2 (9.78)	51.5 (7.41)	51.8 (3.75)	50 (14.8)	72.3 (6.03)	70.5 (8.94)
AGE (years)-median[IQR]	44 [35.8-49]	52 [46.2-57]	52 [49-55]	46.5 [39-63.8]	72 [68-76.8]	71 [64.8-77.5]
APOE4-/APOE4+	68/23 (74.7/25.3%)	7/1 (87.5/12.5%)	13/4 (76.5/23.5%)	39/7 (84.8/15.2%)	24/37 (39.3/60.7%)	10/10 (50/50%)
Plasma p-tau212 n	92	8	17	46	62	20
Plasma p-tau212 range	0.0435-1.69	0.666-2	0.404-2	0.0163-0.444	0.079-1.65	0.174-1.23
Plasma p-tau212 mean (SD)	0.404 (0.377)	1.11 (0.445)	0.929 (0.46)	0.166 (0.0856)	0.498 (0.28)	0.629 (0.29)
Plasma p-tau212 median[IQR]	0.245 [0.156-0.529]	0.926 [0.831- 1.24]	0.764 [0.627-1.23]	0.151 [0.113-0.235]	0.427 [0.33-0.632]	0.65 [0.372-0.738]
Plasma p-tau181 range	3.34-47.8	17-50.1	13.5-42.9	3.95-41.6	4.97-53.7	11.4-37.8
Plasma p-tau181 mean (SD)	15.9 (10.9)	29 (11.8)	23.5 (8.18)	11.1 (7.03)	22.3 (11.5)	22.6 (7.01)
Plasma p-tau181 median[IQR]	11.7 [7.54-20.9]	24.7 [20.4-35.3]	21.6 [18.9-27.7]	9.02 [7.07-12.7]	19.3 [14.6-27.9]	22.5 [18.2-27.4]
Total CAMCOG-DS score- n	73	3	12	0	0	0
Total CAMCOG-DS score- range	25-102	6-56	32-77	-	-	-
Total CAMCOG-DS score- mean(sd)	70.7 (20)	37.7 (27.5)	49.8 (13)	-	-	-
Total CAMCOG-DS score- median[IQR]	74 [57-87]	51 [28.5-53.5]	52 [38.5-57.2]	-	-	-

aDS – asymptomatic Down syndrome; pDS – prodromal Down syndrome; dDS – dementia Down syndrome; CN – cognitively normal; MCI-AD – mild cognitive impairment due to Alzheimer’s Disease; AD – Alzheimer’s disease dementia; IQR – interquartile range; SD – standard deviation. CAMCOG-DS The Cambridge Cognitive Examination adapted for individuals with Down Syndrome