University of Dundee

Post-mortem AT-8 reactive tau species correlate with non-plaque Aβ levels in the frontal cortex of non-AD and AD brains

Malik, Nauman; Miah, Mohi-Uddin; Galgani, Alessandro; McAleese, Kirsty E.; Walker, Lauren; LeBeau, Fiona E. N.

Document Version
Early version, also known as pre-print

Link to publication in Discovery Research Portal

Citation for published version (APA):
Malik, N., Miah, M.-U., Galgani, A., McAleese, K. E., Walker, L., LeBeau, F. E. N., Attems, J., Outeiro, T. F., Thomas, A. J., & Koss, D. (2023). Post-mortem AT-8 reactive tau species correlate with non-plaque Aβ levels in the frontal cortex of non-AD and AD brains. BioRxiv. https://doi.org/10.1101/2023.09.27.559720

1 **Post-mortem AT-8 reactive tau species correlate with non-**

2 **plaque Aβ levels in the frontal cortex of non-AD and AD**

3 **brains**

4

5 **Authors:** [$]Nauman Malik[1], [$]Mohi-Uddin Miah[1], Alessandro Galgani[1], Kirsty McAleese[1],

6 Lauren Walker[1], Fiona E. LeBeau[2], Johannes Attems[1], Tiago F. Outeiro[1,3,4], Alan Thomas[1],

7 David J. Koss[1+5]*

8

9 $= these authors contributed equally.

10   1. Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle

11   University, UK.

12   2. Biosciences, Faculty of Medical Sciences, Newcastle University, Newcastle-upon-

13   Tyne, UK.

14   3. Department of Experimental Neurodegeneration, Center for Biostructural Imaging of

15   Neurodegeneration, University Medical Center Goettingen, Göttingen, Germany

16   4. Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

17   5. Division of Cellular and Systems Medicine, School of Medicine, University of Dundee,

18   Dundee.

19 *Corresponding author= David J. Koss (dkoss001@dundee.ac.uk).

20

21

22

23

24

25

26

27

28 **Abstract**

29 The amyloid cascade hypothesis states that Aβ and its aggregates induce pathological changes in tau,

30 leading to formation of neurofibrillary tangles (NFTs) and cell death. A caveat with this hypothesis is

31 the temporo-spatial divide between plaques and NFTs. This has been addressed by the inclusion of

32 soluble species of Aβ and tau in the revised amyloid cascade hypothesis, however, the demonstration

33 of a correlative relationship between Aβ and tau burden in post-mortem human tissue has remained

34 elusive. Employing frozen and fixed frontal cortex grey and associated white matter tissue from non-

35 AD controls (Con; n=39) and Alzheimer's diseases (AD) cases (n=21), biochemical and

36 immunohistochemical measures of Aβ and AT-8 phosphorylated tau were assessed. Native-state dot-

37 blot from crude tissue lysates demonstrated robust correlations between intraregional Aβ and AT-8

38 tau, such increases in Aβ immunoreactivity conferred increases in AT-8 immunoreactivity, both when

39 considered across the entire cohort as well as separately in Con and AD cases. In contrast, no such

40 association between Aβ plaques and AT-8 were reported when using immunohistochemical

41 measurements. However, when using the non-amyloid precursor protein cross reactive MOAB-2,

42 antibody to measure intracellular Aβ within a subset of cases, a similar correlative relationship with

43 AT-8 tau as that observed in biochemical analysis was observed. Collectively our data suggests that

44 accumulating intracellular Aβ may influence AT-8 pathology. Despite the markedly lower levels of

45 phospho-tau in non-AD controls correlative relationships between AT-8 phospho-tau and Aβ as

46 measured in both biochemical and immunohistochemical assays were more robust in non-AD

47 controls, suggesting a physiological association of Aβ production and tau phosphorylation, at least

48 within the frontal cortex. Such interactions between regional Aβ load and phospho-tau load may

49 become modified with disease potentially, as a consequence of interregional tau seed propagation,

50 and thus may diminish the linear relationship observed between Aβ and phospho-tau in non-AD

51 controls. This study provides evidence supportive of the revised amyloid cascade hypothesis, and

52 demonstrates an associative relationship between AT-8 tau pathology and intracellular Aβ but not

53 extracellular Aβ plaques.

54

55

56

57

58

59

Introduction

The original amyloid cascade hypothesis stated that the extracellular deposition of insoluble beta-amyloid (Aβ) plaques drives intracellular tau phosphorylation, the formation of neurofibrillary tangles (NFTs), and the subsequent neurodegeneration which underlies the pathology of Alzheimer's disease (AD) [1]. Owing to the lack of correlation between plaque burden and cognitive impairment, as well as a growing understanding of the toxicity of fibrillar and pre-fibrillar intermediate species, the hypothesis has been revised to include roles for Aβ oligomers and tau oligomers[2]. Whilst the recent outcomes of plaque clearing and Aβ oligomer targeted immunotherapies[3-5] support this revised amyloid cascade hypothesis, several inconsistencies relating to the interaction of Aβ and tau remain.

Foremost, is the spatio-temporal disconnect between the emergence and progression of Aβ plaque and tau NFT pathology. Based on the post-mortem neuropathological Thal phases of Aβ deposition and positron emission tomography (PET) imaging studies Aβ plaques originate within the neocortex, specifically within the orbito-frontal and medial parietal cortices, before spreading to the hippocampus, the brain stem and cerebellum[6, 7]. In contrast, as reflected by Braak NFT staging, tau pathology initially occurs in the entorhinal cortex and hippocampus and subsequently spreads to the lateral temporal and parietal cortices and finally to the frontal and occipital cortices[8, 9].

Cross sectional population studies further highlight the independent nature of the two hallmark pathologies, reporting that tau pathology consistent with Braak Stages I-II, occurs more readily with age than that of plaque depositions[10]. Consequently, Aβ deposition is not a prerequisite for NFT formation in aging, nor in the cases of primary tauopathies[11]. The independence of tau pathology from Aβ plaques is particularly evident in cases of primary age-related tauopathies (PART), which present with AD related NFTs in a spatial pattern consistent with Braak NFT stages up to stage IV without Aβ plaque deposition[12]. Moreover, the demonstration of prion-like spreading via tau seed templating and pathology propagation, provides a mechanistic process by which the presence of tau pathology may occur independent from the influence of Aβ plaques[13]. Such tau seed propagation of pathology likely contributes to the progression of tau pathology in many tauopathies, including AD.

Taken together, the direct causation of NFTs, purely as a consequence of Aβ plaque burden is difficult to ratify, with the differential emergence in time and space of the neuropathological hallmarks, as well as the independent occurrence of tau aggregations in other neurodegenerative conditions.

However, it remains likely that plaque deposition or rather the process of amyloid plaque formation, influences the generation of tau pathology. This is perhaps most strongly supported by numerous biochemical studies of human brain tissue, which report robust correlations with pathological Aβ and

92   tau species[14-17]. Despite the close relationship between tau and Aβ levels in various biochemical

93   assays, histochemical approaches frequently fail to detect such correlations. The disconnect between

94   biochemical and histochemical analysis clearly highlights differences in the pathological species

95   measured within the different methodological approaches.

96   In line with the revised amyloid cascade hypothesis[2], a range of experimental models demonstrate

97   that soluble pathological species of both tau and Aβ exert toxic influence within the brain, evident in

98   injection models [18-21], familial AD (FAD) [22-24], tauopathy[25-27] mouse models and cell culture

99   approaches[28, 29]. Whilst APP centric FAD mouse models, do not develop NFTs, there is clear

100   evidence of increased tau phosphorylation within these models. Moreover in multi-genic mice, in

101   which a human mutant tau gene is included, APP centric mutations can accelerate and enhance NFT

102   pathology[30]. Nevertheless, evidence for the induction of tau pathology following Aβ intracerebral

103   delivery is sparse in non-transgenic animals[31, 32] and even in tau transgenic mice[33].

104   Whilst there are several possible explanations for the failure of exogenous Aβ to drive tau pathology

105   in-vivo, one possible contributing factor is that the sole delivery of Aβ to the extracellular space may

106   not be sufficient to drive tau pathology. Indeed, a growing body of evidence suggests that intracellular

107   Aβ accumulation may also play a role in the pathobiology of AD[34], influencing cellular

108   dysfunction[35-37] and tau phosphorylation[22].

109   Despite the potential for non-plaque Aβ to contribute to the production of tau pathology, few studies

110   have sought to examine both biochemical and immunohistochemical quantification of Aβ and tau

111   pathology within the same cases, thus, allowing for a direct comparison between the correlative

112   strength of total Aβ, plaque Aβ and intracellular Aβ with tau pathology. This current study aimed to

113   quantify such parameters across cases to establish potential correlative relationships between

114   pathological species in order to further understand the influence that Aβ, in various forms, may have

115   upon the regional generation of tau pathology, both in neuropathologically confirmed cases of AD as

116   well as non-AD control cases.

117

118   Methods

119   Human post-mortem brain tissue

120   A study cohort of post-mortem human brains from clinico-pathologically classified AD (n=21) and non-

121   neurodegenerative control cases (Con, n=39) was obtained from the Newcastle Brain Tissue Resource

122   (NBTR). AD Subjects had been clinically assessed during life prior to brain tissue donation and

123   diagnosed with dementia due AD. Control cases similarly had been assessed during life and at the time

124 of death did not have dementia. The final clinico-pathological diagnoses were established by

125 combining clinical neuropathological data reviewed at regular meetings involving JA and AT.

126 Neuropathological diagnoses were based on assessment of brain tissue according to the National

127 Institute of Ageing – Alzheimer's Association (NIA-AA) criteria[38], including Braak NFT staging[39],

128 Thal phases [6], and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scoring[40],

129 as well as Braak LB stages[41], and Newcastle / McKeith Criteria [42, 43] (Table 1 and S1 for full

130 details).

131 For histology and tissue micro-array (TMA; see below), tissue sections were prepared from the right

132 hemisphere of the brain and fixed for 4-6 weeks in 4% paraformaldehyde. Corresponding frozen

133 frontal grey (GM) and white matter (WM) tissue (Brodmann's area (BA) 9) was obtained from the left

134 hemisphere, dissected in a coronal plane and snap frozen between copper plates at -120°C prior to

135 being stored at -80°C. Due to limitations in tissue availability, it was not possible to obtain both fixed

136 and frozen tissue for all cases (see Table 1 and S1 for full details). Comparative analysis of age and

137 post-mortem interval (PMI) between disease groups determined no significant difference in either

138 measure ($p > 0.05$).

139

140 **Tissue lysis**

141 ~250mg of frozen frontal tissue was electronically homogenised 1:10 (W/V) in 0.2M tetraethyl

142 ammonium bicarbonate (TEAB, pH 7.2, Sigma) with 1% SDS, containing protease (1 per 10ml,

143 Complete , Roche) and phosphatase inhibitors (1 per 10mls, PhosSTOP, sigma) using an Ultra-turrax

144 T10 homogeniser (5 mm diameter probe; 30,000rpm) for 15 sec. Lysate were aliquoted and stored at

145 -80°C, prior to use.

146

147 **Immunoblot quantification of AD markers:**

148 Dot blots were conducted for total Aβ and AT-8 phospho-tau in both grey and white matter samples.

149 The protein concentration of GM and WM crude lysate were adjusted to 0.5μg/μl as per Bradford assay

150 and dotted directly to a nitrocellulose membrane at 10μl (5μg/dot) and left to dry for 20 minutes

151 before further processing. The membranes, briefly washed in Tris-buffered saline (TBS; in mM; 50

152 Trizma base, 150 NaCl, pH= 7.6) prior to being blocked in 5% milk powder containing Tris-Buffered

153 Saline with 0.1% Tween 20 (TBST) at room temperature for 1 hour. After blocking, blots were rinsed

154 in TBS washing buffer 3 times for 5 minutes each. Membranes where subsequently placed in primary

155 antibody solution (TBST, 5% bovine serum albumin and 0.05% sodium-Azide) containing either MOAB-

5

156 2 (1:1000, Cat# M-1586-100, Biosensis) for the detection of Aβ or AT-8 (1:1000, Cat# AB_223647,

157 Thermofisher) for phospho-tau and incubated overnight at 4°C. The membranes were then washed

158 in TBST before being incubated for 1 hour at room temperature in horse radish peroxidase conjugated

159 goat anti-mouse secondary IgG antibody (TBST+5% milk powder+1:5000 dilution) prior to repeated

160 washing before being development. Immunoreactivity was visualized via enhanced

161 chemiluminescence (1.25 mM Luminol, 25μl of 3%H2O2 and 50μl coumaric acid was incubated for 1

162 minute). The signal was captured by using digital western blot camera, with high sensitivity and auto

163 exposure being selected. The images were saved as 8- bit for illustration and 16-bit for quantification.

164 Total protein loading was determined via Ponceau S general protein stain (0.1% Ponceau S (w/v) and

165 5.0% acetic acid (w/v) in ddH$_2$O water) and resulting loading staining captured.

166 **Immuno-blot quantification**

167 Immunoreactivity of enhanced chemiluminescent (ECL) luminol blots and Ponceau S-stained blots was

168 quantified from 16-bit digitized images based on area under the curve measurements as computed by

169 ImageJ (Ver 1.53e, NIH, USA). Normalization of immunoblot intensity values were then performed

170 using total protein adjusted values. The 52 samples of human frontal cortex GM and WM were

171 processed in 4 separate batches and each batch normalized to the mean value of control cases (each

172 blot containing >3 Br 0-IV control cases) prior to pooling values between blots.

173

174 **Immunohistochemical quantification of Aβ plaques and phospho-tau (AT-8)**

175 Regional quantification of the Aβ plaque and AT-8 phospho-tau load within the frontal cortex (BA9)

176 via TMA slides, as described previously[44]. Sections (6 μm, thick) were cut from paraffin embedded

177 TMA blocks tissue blocks comprising of cylindrical tissue cores taken from multiple brain region

178 specific blocks and mounted on glass slides. Slides containing a 3 mm diameter samples of BA9 frontal

179 cortex were baked at 60°C for 1hr prior to being dewaxed in xylene, rehydrated in descending

180 concentration of ethanol (5 mins immersion) and washed in TBS. Slides intended for phospho-tau

181 staining were treated with microwave assisted antigen retrieval (800 W, 10 mins) in citrate buffer (10

182 mM Citric acid, 0.05 % Tween 20, pH 6) and those intended for Aβ plaque staining were submerged in

183 90% Formic acid for 1hr at RT, before endogenous peroxidases were quenched in H$_2$O$_2$ (3%, 20 mins

184 submersion). Following consecutive washes in TBS and TBST, slides were incubated with either mouse

185 4G8 (1:16000, Cat# SIG-39200, Covance) or anti-AT-8 (1:4000) in TBS for 1 hr and immunoreactivity

186 visualised via the MENAPATH HRP polymer detection kit (Menarini diagnostics, Wokingham, UK) and

187 3,3'- Diaminobenzidine (DAB) chromogen with appropriate TBS and TBST washes performed between

6

188   steps. Slides were co-stained with haematoxylin prior to being dehydrated in ethanol, cleared in

189   xylene and mounted in dibutylphthalate polystyrene xylene (DPX).

190   Stained BA9 frontal cortex samples were imaged at x100 magnification with a semi-automated

191   microscope (Nikon Eclipse 90i microscope, DsFi1 camera and NIS elements software V 3.0, Nikon). For

192   each case, multiple images were captured to form a 3x3 image grid with 15% overlap in adjacent

193   images, such that an area of 1.7mm was sampled from each case.

194   Following visual quality control inspection and the application of regions of interest (ROI) to exclude

195   areas of tissue folds and tears, a consistent restriction threshold for 4G8 (R50-180, G20-168, and B8-

196   139) and AT-8 (R25-170, G27-156, B11-126) was applied producing a binary signal image from which

197   the percentage area of immunoreactivity could be acquired. For the quantification of Aβ plaques, 4G8

198   images were further processed by means of size exclusion, restricting object detection to >100μm^2,

199   thus avoiding inclusion of intracellular APP and Aβ.

200

Immuno-fluorescent histochemical analysis of intracellular Aβ and phospho-Tau (AT-8)

202   Paraffin embedded tissue blocks of the frontal cortex BA9 were used to prepare sections (6 μm thick)

203   for the purpose of multiplex intracellular Aβ and phospho-tau fluorescent staining. Slide mounted

204   frontal cortex sections were baked at 60°C for 1hr, dewaxed and rehydrated and subjected to antigen

205   retrieval in citrate buffer and formic acid treatment (as above). Slides were then blocked in TBST

206   containing 10% normal goat serum for 1hr at RT and incubated in mouse IgG2b anti-MOAB-2 and

207   mouse IgG1 anti-AT-8 (1:500, for both) overnight at 4°C, prior to incubation in secondary antibodies

208   (goat-anti mouse IgG1 – Alexa 488 and goat-anti mouse IgG2b-Alexa 594, 1:1000 for both, Invitrogen).

209   Endogenous tissue fluorescent was quenched via post-staining treatment with Sudan Black (0.01%,

210   70% ethanol, 5mins submersion) before slides were coverslipped with DAPI-containing Prolong

211   Diamond Mounting media (Fisher Scientific). In a subset of slides, the limited co-localization of MOAB-

212   2 labelled Aβ and APP was established, staining sections with mouse- IgG2b anti-MOAB-2 and Rabbit-

213   anti-APP (1:500, Cat# ab15272, abcam) and appropriate secondary antibodies. Fluorescence antibody

214   labelled sections were imaged via a wide-field fluorescence microscope system (Nikon Eclipse 90i

215   microscope, DsQi1Mc camera and NIS elements software V 3.0, Nikon).

216   One section per case was examined at 400x magnification with 3 images per grey matter and white

217   matter region selected at random. As these images were used for quantification of intracellular Aβ,

218   excluding Aβ-plaques, any region selected which contained multiple plaques was excluded and

219   another region selected. ROI were manually applied to each image and folds, tears and plaques were

7

220 excluded, before images were converted to grey scale and a consistent threshold applied to generate

221 a binary image from which percentage area of immunoreactivity was determined. The mean

222 percentage area of immunoreactivity was calculated per grey and white matter area per case.

223

224 **Data analysis**

225 Data were subjected to Shapiro-Wilk normality test for normal distribution, prior to statistical

226 comparison between control and AD cases using a non-parametric Mann-Whitney U test (GraphPad

227 Prism Ver. 5).   In SPSS, two-tailed Spearman's correlation was used for correlation analysis. Given the

228 association of increasing Braak stage with age, all correlations with Braak staging were performed with

229 partial correlations controlling from age. A series of one- tailed t-test were performed to identify the

230 initial stage at which measures were significantly elevated from Braak 0 pathological controls. For all

231 analysis, $p<0.05$ was considered as statistically significant, with increasing statistical reliability for

232 $p<0.01$, $p<0.001$ and $p<0.0001$.

233

234 **Results**

235 **Biochemical analysis of Aβ and phospho-tau pathology.**

236 In order to limit the potential confounding influence of age-related Aβ independent tau pathology as

237 seen in PART[12], Brodmann's area 9 of the superior frontal cortex, a region which does not develop

238 NFTs until late stage of AD related disease progression (Braak NFT V-VI) was selected for investigation.

239 Using a native state dot-blot quantification, crude tissue lysates of grey matter of this region as well

240 as the associated white matter of AD (n=17) and non-AD controls (n=35), were probed for Aβ via the

241 non-APP cross-reactive MOAB-2 antibody and for tau pathology using the phospho-tau specific

242 antibody AT-8.

243 When considered purely based on the neuropathological diagnosis of either non-AD and AD, levels of

244 AT-8 phospho-tau were elevated in AD cases, both in GM (13.24±3.3-fold cf. non-AD, $p<0.001$, fig1 a.i

245 + b.i) and WM (9±2.6 fold cf. non-AD, $p<0.001$, fig1 a.i + b.i). Despite a numerically higher mean within

246 the GM compared to the WM, there was no statistically significant difference between the magnitude

247 of increase between GM and WM in AD cases ($p>0.05$). Similarly, when Aβ levels were examined based

248 on the neuropathological cohort stratification of Non-AD and AD, elevations were apparent within the

249 GM (3.35±0.59 fold c.f non-AD, $p<0.01$, fig1 a.ii + c.ii) and WM (2.56±0.56 fold c.f non-AD, $p<0.05$, fig1

250 a.ii + b.ii) of AD cases. Again, no difference between the magnitude of increase within AD cases relative

251 to control cases was observed between GM and WM ($p>0.05$).

252   Such an outcome from the analysis of phospho-tau and Aβ between AD and non-AD cases is not

253   surprising but serves to validate the use of dot-blots to measure biochemical changes in phospho-tau

254   and Aβ.

255   To further place the observed changes of tau and Aβ within the context of disease progression, the

256   cohort was subdivided into their respective Braak stages (0-VI) and the association of crude lysate

257   measures of AT-8 phospho-tau and Aβ with disease progression was determined (Fig 1 c.i + ii).

258   Phospho-tau AT-8 immunoreactivity correlated with increasing Braak stages when considered across

259   the entire cohort within the GM (r=0.7, p<0.001) as well as in the WM (r=0.67, p<0.001). Additionally,

260   a modest but significant correlation between Braak NFT stage and age was reported (r=0.34, p<0.05)

261   although age did not correlate with biochemical measures of AT-8 (p>0.05, data not shown).

262   Correlations between AT-8 immunoreactivity and Braak NFT remained when adjusting for age (r=0.62

263   and r=0.57 for GM and WM respectively, p<0.001 for both, fig 1 c). Interestingly when probed for the

264   stage at which phospho-tau levels were significantly elevated from that of "pathologically-free" Braak

265   stage 0 cases, Braak stage IV was indicated (p<0.05, for both GM and WM). Braak stage IV being the

266   stage prior to gross affection of the frontal cortex with NFTs. Furthermore, when split according to

267   neuropathological diagnosis, and controlled for age, a significant correlation was observed in the non-

268   AD control group in GM (Fig 1 c.i, r=0.47, p<0.01) and WM (Fig 1c.i, r=0.43, p<0.05), but not in AD

269   cases (Fig 1 c.i, p>0.05). Together the data suggest that in non-AD control cases, AT-8 phospho-tau

270   increases within the frontal cortex in line with the intra-regional spatial AT-8 positive NFT progression,

271   somewhat independent of age. Thus, initial levels of frontal cortex AT-8 positive phospho-tau may be

272   regionally generated.

273   Following a similar line of investigation for the accumulation of Aβ in relation to disease progression,

274   Aβ levels were correlated with individual Braak NFT stages. Across the entire cohort, robust

275   correlations were reported for both the GM (r=0.67, p<0.001, fig 1 d) and WM (r=0.6, p<0.001, fig 1

276   d). Again, correlations remained when controlling for age (r=0.54 and r=0.53 for GM and WM

277   respectively, p<0.001 for both). In line with observations of AT-8 phospho-tau, individual comparisons

278   with Braak 0 cases reported an initial significant elevation from the "pathologically-free" baseline at

279   Braak 4, in GM and WM samples (p<0.05). Consistent with similar measures of AT-8 phospho-tau,

280   when divided into non-AD and AD categories, increasing total Aβ correlated with progressive Braak

281   stages in the non-AD group in both the GM and WM (r= 0.36, p<0.05 and r=0.46, p<0.01 in GM and

282   WM respectively, fig 1 d) and not in the AD group (p>0.05). Similar to correlation with Braak staging,

283   all other neuropathological classifications also reported robust correlations when considered as a

284   single cohort (See S.table 2). When considering Non-AD controls only, significant correlation with AT8

285   phospho-tau was only apparent with CERAD scores for GM and WM (r=0.4, p<0.05 and r=0.47,

286   p<0.001, respectively) whilst GM and WM scores for Aβ correlated with Thal, CERAD and NIA-AA

287   (r=0.38-0.52, p<0.05, see S.table 2 for full details). In terms of AD cases, correlations were apparent

288   for all biochemical measures with all neuropathological scheme (see S.table 2).

289   Collectively, the data suggest a robust relationship of AT-8 and Aβ with disease progression within the

290   GM and WM of the frontal cortex, occurring not only in confirmed cases of AD but also non-AD

291   controls. In the frontal cortex, intra-regional pathology progresses in accordance with global AD-

292   related brain tau pathology as quantified by Braak NFT stages and this was most evident in non-AD

293   controls.

294   Independent of global brain AD related pathological changes, a critical element to regional pathology

295   is to determine whether biochemical measures of tau pathology and Aβ correlate on a case-by-case

296   basis, as the disconnect of histochemical tau and Aβ pathological hallmarks has long been a major

297   caveat to the amyloid cascade hypothesis. Indeed, a robust correlation between biochemical

298   measures of AT-8 phospho-tau and total Aβ measures was observed when considered as a single

299   cohort (Non-AD + AD cases) in the GM (r=0.75, p<0.001, fig 1 d.i) and in the WM (r=0.67, p<0.001, fig1

300   e.i). Remarkably, when examined separately within Non-AD controls and AD cases, correlations

301   between AT-8 phospho-tau and Aβ were apparent in both control cases (GM; r=0.52, p<0.001 and

302   WM; r=0.67, p<0.001, fig 1 d ii + e ii) as well as AD cases (r=0.5, p<0.05 and r=0.73, p<0.01 in GM and

303   WM, fig 1 d iii + e iii).

304

305   **Histochemical quantifications of AT-8 phospho-tau and Aβ plaques.**

306   In order to establish if the biochemical derived relationship of increased in Aβ immunoreactivity

307   conferring increased in AT-8 immunoreactivity was primarily driven by an association of Aβ plaques

308   with AT-8 phospho-tau, semi-quantitative immunohistochemistry analysis was performed (Fig 2 a, and

309   see S.table 1 for details). Based on % area coverage, AT-8 immunoreactivity, a composite of NFTs and

310   NTs reported a marked ~ 100 fold increase in % coverage in the frontal grey matter of AD cases

311   compared to controls (7.6±2.5% vs. 0.07±0.02%, in AD and non-AD cases respectively, p<0.001, fig 2

312   b.i). Equally, quantification of the % area coverage of Aβ plaques between AD cases and non-AD

313   controls, also, unsurprisingly reported a significant increase with the AD cases (14.26±1.7% cf.

314   3.55±1%, p<0.001, fig 2 b.ii).

315   When measures were considered in relation to progressive Braak NFT staging, AT-8 phospho-tau

316   (r=0.55, p<0.001, fig 2 c.i) and Aβ plaques (r=0.67, p<0.001, fig 2 c.ii) strongly correlated with Braak

317   stage, following a correction for age. Again, a significant elevation in AT-8 phospho-tau and Aβ plaque

318 coverage from "pathologically-free" Braak stage 0 cases was reported at Braak Stage IV (p<0.05), in

319 line with observations from biochemical measurements. When controlling for age, no significant

320 correlations were observed for AT-8 phospho-tau or Aβ plaques for either non-AD or AD groups

321 (p>0.05). Further analysis with additional neuropathological classification reported strong correlations

322 with Thal and CERAD and NIA-AA when considered as a single cohort and in control only (S.table 3).

323 Collectively, the data largely suggests that correlative relationships reported within the overall cohort

324 likely stems from group effects driven by the general increase of pathological hallmarks between non-

325 AD and AD groups and not as incremental regional increase in line with progressive Braak stage. Such

326 observations contrast with the findings of the biochemical investigation.

327 Equally correlations between IHC quantified Aβ and AT-8 phospho-tau also reported a correlation only

328 when the data were analysed as a single cohort combining non-AD controls and AD cases (r=0.65,

329 p<0.001, fig 2 d) and not when examined as a separate data set of non-AD controls or AD cases

330 (p>0.05, for both, fig 2 dii+iii). Nevertheless, biochemical measures correlated with respective

331 histochemical measures for AT-8 and Aβ (see Stable 4). Thus, despite the contradiction between

332 biochemically measured association of AT-8 phospho-tau and total Aβ and histologically measured AT-

333 8 phospho-tau and Aβ plaque load, the measures by differing methodological means are somewhat

334 inter-related.

335

336 **Quantification of intracellular Aβ**

337 The absence of a correlation between IHC measures AT-8 and plaques (fig 2), despite a robust

338 correlation with of biochemical AT-8 and total Aβ measures from crude tissue lysates (fig 1), suggests

339 the possible inclusion of additional Aβ sources within the biochemical quantification. Accordingly, the

340 application of MOAB-2 Aβ antibody to fixed post-mortem human brain tissue sections, labelled both

341 extracellular plaques and intracellular pools of Aβ (fig 3). Comparisons of APP and MOAB-2 Aβ

342 labelling, demonstrated a clear distinction in subcellular and plaque labelling in both GM (fig 3 a) and

343 WM (fig 3 b). Whilst there is a degree of overlap as would be expected given the spatial limitation and

344 the production of Aβ from APP, co-localisation is absence in the majority of puncta (fig 3; white

345 arrows), confirming the specificity of MOAB-2 for the labelling of Aβ, as previously reported[45, 46].

346 Across a subset of the cohort, both in the GM and WM, a progressive increase in intracellular Aβ

347 staining in line with increase AT-8 immunoreactivity was observed (fig 4.a). When intracellular Aβ was

348 quantified according to % area coverage, a significant increase in the levels was observed in AD cases

349 compared to controls (fig 4b.i, p<0.001 in GM and p<0.05 in WM). Similarly, AT-8, as measured by

350 immunofluorescence was again elevated in AD cases (fig 4 b ii, p<0.001 in GM and WM). Both AT-8

351   (r=0.62, p<0.01 in GM and r=0.5, p<0.05 in WM) as well as intracellular Aβ (r=0.61, p<0.01 in GM)

352   correlated with Braak stage when controlling for age. Equally, strong correlations were observed with

353   Thal, CERAD and NIA-AA also (see S.table 5). No correlations with any neuropathological classifications

354   were reported when the cohort was spilt according to Non-AD and AD groups. Nevertheless,

355   correlative measures between intracellular Aβ and AT-8 phospho-tau reported a significant

356   relationship across all cases (in GM, r=0.76 and in WM, r=0.71, p<0.01 for both, fig 4 c i + d i,) and in

357   control cases only (in GM, r=0.82 and in WM, r=0.76, p<0.01 for both, fig 4 c ii + d ii). Interestingly

358   when considering only the AD cases, a significant inverse correlation of intracellular Aβ with AT-8

359   phospho-tau in the GM, was apparent (r=-0.74, p<0.05, fig 4 c iii), although no correlation was

360   reported in the WM (fig 4 d iii). As with IHC quantifications of plaques and AT-8 as per TMA slides,

361   measures of intracellular Aβ and AT-8 here, also correlated with biochemical measures when

362   considered as a single group, although no significant correlations were observed when split into

363   Control and AD categories (S. table4).

364   Collectively quantification of the intracellular Aβ and its correlation with AT-8 phospho-tau appears

365   to support the biochemical findings of a close relationship between AT-8 and Aβ, particularly in non-

366   AD control cases. However is should be noted that spatial colocalization of AT-8 and intracellular Aβ

367   was not common and appeared to be the exception rather than the rule (fig 5), instead here we find

368   a correlation of regional AT-8 burden with regional intracellular Aβ.

369

Discussion

371   Collectively this study reports an apparent correlation between biochemical Aβ and AT-8 phospho-tau

372   measures. Such a relationship was not reproduced when comparing IHC based quantification of Aβ

373   plaques and AT-8 phospho-tau but was observed when considering IHC measures of phospho-tau and

374   intracellular Aβ. These correlative relationships are perhaps surprisingly strongest within the non-AD

375   control group and are evident in both grey and white matter. Together the data suggests a close

376   relationship between non-plaque Aβ and tau, which is at least partially due to the accumulation of

377   intracellular Aβ and its potential influence on tau phosphorylation.

378

379

380

Regional correlation of tau and Aβ

12

382   A long-standing critique of the amyloid cascade hypothesis has been the disconnect between NFT and
383   Aβ-plaque burden within a given brain region of either non-AD controls or indeed AD cases [47, 48].
384   However many biochemical approaches have previously found correlations between Aβ, either total
385   Aβ or specifically Aβ1-42, with a range of phosphorylated and oligomeric tau markers within the GM
386   of a given cortical region[14-17]. Furthermore, disease dependent changes in white matter Aβ levels
387   have also been previously observed using $Aβ_{40}$ and $Aβ_{42}$ ELISAs[49] and like-wise hyperphosphorylated
388   tau has also been observed biochemically within the white matter of AD cases[13, 50]. Here we
389   observed that levels of AT-8 reactive phospho-tau and Aβ increased in non-AD controls in line with
390   Braak stage progression, in both the frontal grey and white matter and exhibited a positive correlation
391   between Aβ levels and AT-8 when measured biochemically.   Whilst our observations cannot
392   determine causality, our findings are consistent with many *in-vitro* experiments in which the
393   application of Aβ to various cellular preparations results in downstream tau phosphorylation[28, 51,
394   52]. Moreover, support for the interaction of Aβ with tau pathology, can be gained from studies
395   reporting that interventions targeting Aβ levels consequently reduce tau pathology both *in-vitro* and
396   *in-vivo* models[53, 54] as well as in biofluids obtained from human clinical trials[3, 4, 55].

397   When considering the association of Aβ and tau phosphorylation within non-AD controls, our findings
398   are similar to the observation of a previously seen correlation between $Aβ_{1-40}$ level and p-181 tau in
399   the CSF of control cases[56]. However here we can extend this finding to show that within the frontal
400   cortex there is a regional dependence between Aβ levels and tau pathology. As work by others have
401   reported no or minimal seed competent tau within the frontal cortex in non-AD controls [50], the
402   source of pathology within the frontal cortex is likely to mainly be intraregional, with minimal
403   influence of extra-regional spread.   Such intraregional dependence would be consistent with the
404   strong correlation between Aβ and AT-8 signals within the non-AD controls, which is somewhat
405   weakened in AD cases, when seed competent tau species have presumably invaded the frontal
406   cortex[13], the self-propagation of tau pathology thus diminishing the relative contribution of Aβ to
407   the production of phospho-tau species. Interestingly, a linear relationship between regional soluble
408   tau phospho-species and $Aβ_{1-42}$ has previously been reported alongside a bimodal relationship within
409   the insoluble fraction, implying a weakening of the relationship between $Aβ_{1-42}$ and tau
410   phosphorylation once aggregated [17]. Thus, given that this present study did not seek to distinguish
411   between soluble and insoluble pathology, the increased representation of insoluble tau pathology
412   within lysates from AD cases, likely further explains the weakening of the linear relationship between
413   Aβ and tau phosphorylation in AD cases.

414   Accordingly, no linear correlation was observed between plaque load and AT-8 load when measured
415   histologically. Such differences may relate to the presumed loss of extracellular soluble Aβ and

13

416   exclusion of intracellular Aβ pools, as is common practice when assessing Aβ burden as part of a

417   neuropathological assessment[44].

418

419   **Intracellular Aβ**

420   Historically, intracellular Aβ has been problematic in its quantification, largely due to the cross-

421   reactivity of Aβ antibodies with APP and other intermediate APP metabolites. Howecer, several

422   commercial Aβ antibodies are available, including MOAB-2 which shows no cross-reactivity with APP

423   under many conditions [45, 46].

424   Although often overlooked, the production of Aβ occurs intracellularly following endosomal APP

425   cleavage via β-secretase [57] and sequential γ-secretase processing within either Golgi [58] or

426   lysosomal [59] compartments. Whilst the majority of Aβ is trafficked to the extracellular space, age-

427   related changes in the relative production of Aβ peptide length [60] and disease alterations to

428   trafficking mechanisms, such as Rab GTPases [61, 62], may act synergically to enhance the retention

429   of intracellularly produced Aβ and or indeed its reuptake, leading to its intracellular accumulation [63].

430   Accordingly, post-mortem examination of the entorhinal cortex and hippocampus of non-diseased

431   non-AD cases, suggests an increase in intracellular Aβ in line with increasing age [64, 65] and

432   furthermore AD animal models also show an age-related accumulation of intracellular Aβ [46, 66].

433

434   **Intracellular Aβ and tau**

435   Here, when selectively measuring intracellular Aβ, a positive correlation between Aβ and AT-8 in the

436   frontal cortex of non-AD controls was observed. This is consistent with biochemical measures of

437   frontal cortex lysates in the same cases as present here or indeed previously reported biochemical

438   measures in the lateral temporal cortex of a different study cohort[15]. However, in work by others,

439   no such relationship has been observed in the entorhinal cortex [64][67]. Such a contrast in

440   relationships may relate to the regions selected for investigation. The entorhinal cortex is one of the

441   earliest affected cortical regions with tau pathology in AD, and thus presumably represents a cortical

442   area of increased vulnerability to tau pathology. Such vulnerability may mean that tau pathology may

443   be generated in an Aβ independent manner within the region as is the case of primary age-related

444   tauopathy [12]. Nevertheless, within the prefrontal cortex, a region which does not demonstrate

445   robust age related NFT tau pathology and is not burdened with NFTs until late into the Braak NFT

446   staging criteria (Braak V-VI), such modest pre-tangle tau-pathology generated in this region may be

447   largely dependent on Aβ mediated mechanism. Such a mechanism may become modified under

14

448   pathological conditions, either upon reaching a critical threshold of intracellular Aβ accumulation
449   and/or via seed component invasion.

450   Interestingly within the grey matter, although a positive relationship between intracellular Aβ and
451   phospho-tau was observed in non-AD control cases, an inverse relationship was observed in AD cases.
452   Whilst intracellular Aβ levels remained elevated compared to controls, there has been reports of a
453   reduction of intracellular Aβ levels in line with the deposition of Aβ plaques in mice models [68] as
454   well as in serial observations in Down's syndrome brains [69] and cases of late stage NFT mediated
455   neurodegeneration [67]. Such contradictions may be due to differences in the specific regions of
456   investigations. However, it is equally plausible that elevation of intracellular Aβ precedes and indeed
457   acts as a source for extracellular plaque deposition, with the excessive deposition of plaques at later
458   stages subsequently reducing intracellular Aβ levels as observed in animal model [68]. In turn tau
459   pathology may continue to grow due to the influence of seed component species.

460   In any case, excessive intracellular Aβ accumulation, is unlikely to be benign. The familial Osaka E639Δ
461   APP mutant which produces non-fibril E22Δ Aβ gives rise to an accumulation of intracellular Aβ
462   oligomers in the absence of plaques. In AD patients or mouse models carrying the Osaka mutation,
463   pronounced cognitive impairments, cellular stress, synaptic spine loss and critically pathological tau
464   phosphorylation and conformational changes are observed [22, 45]. Whilst several of the downstream
465   cellular dysfunction may be independent of tau [70], these studies nevertheless highlight the induction
466   of tau pathology via Aβ independent of plaque formation, in support of the observed correlation of
467   intracellular Aβ and phospho-tau observed here.

468   Given emerging evidence from clinical Aβ antibody trials[71], which supporting the targeting of soluble
469   fibrillar Aβ species to consequently reduce tau pathology, further understanding the degree of
470   interaction between Aβ and tau will provide greater insight into the mechanisms of AD related
471   pathogenesis. Equally, in light of the facilitation of fibril seeding by the existence of pre-existing tau
472   phosphorylation/pathology in mice [72-74], the targeting of pre-tangle soluble tau elevations in late
473   stage affected brain regions, may protect against tau seed infiltration as part of AD disease
474   progression, and may provide an effective stalling of the condition.

475

476

477

478

Conclusions

Collectively this study demonstrates the robust correlation of AT-8 reactive tau and Aβ in the frontal cortex of both non-AD controls and AD cases when measured biochemically. Given that such linear increases in Aβ plaques and AT-8 pathology is not observed when quantified via IHC, the study demonstrates the potential influence of non-plaque Aβ in the intra-regional generation of tau pathology. Specifically, the occurrence and accumulation of intracellular Aβ, in line with AD pathological progression, at least in part correlated with AT-8 pathology and thus may contribute to production of tau pathology. This finding is supportive of the amyloid cascade hypothesis, yet in late-stage AD cases such a relationship may be diminished, with additional factors contributing to tau pathology, at least within the frontal cortex. Critically, the observation of a localised relationship between Aβ and phospho-tau in cases with low Braak NFTs stages implies that there is a degree of regionally generated AD related pathology, which may be tolerated within a physiological range. Following the age-related accumulation of pathology, this regionally produced burden may prime the region for the invasion of seed competent forms of tau originating from connected regions, raising the burden beyond a critical threshold, and thus removing the necessity of Aβ for the propagation of tau pathology.

Acknowledgements

509   Legends

510   Table legends

511   **Table 1. Post-mortem human tissue cases and use.** Human cases use for immuno-blots and

512   immunohistochemistry for plaques and AT-8 as well as intracellular Aβ and AT-8 are listed. Case are

513   separated by disease classification according to non-diseased controls (Con) and Alzheimer's disease

514   (AD). Case numbers (n), sex, age, post-mortem interval (PMI), neurofibrillary tangle (NFT) Braak stage,

515   Thal phase, Consortium to Establish a Registry for Alzheimer's Disease (CERAD), the National Institute

516   of Ageing – Alzheimer's Association (NIA-AA) criteria, Lewy body (LB) Braak stage and McKeith criteria

517   are provided. For age and PMI both range and mean ±SEM are provided. For numerical scores of

518   pathology, range and percentage composition are given. For CERAD scores, negative (neg), A and B

519   reported. For NIA-AA, not, low and intermediate (inter) risk for Alzheimer's disease. For McKeith

520   criteria, only percentage composition is given, where cases free of LBs (No LB), brainstem, Limbic and

521   neocortical (Neo) predominate are indicated.

522

523   Figure legends

524   **Figure 1. Biochemical quantification of AT-8 phospho-tau and Aβ in the frontal cortex of non-AD and**

525   **AD cases**. A). Example dot blots of AT-8 (i) and MOAB-2 (Aβ; ii) immunoreactivity and associated

526   ponceau total protein stain, produced from crude tissue lysates of frontal grey (GM) and white matter

527   (WM) in control (Con; black lettering) and Alzheimer's disease (AD; red lettering) cases. Braak NFT

528   stage of each sample is shown. B). Comparison of mean AT-8 (i) and Aβ (ii) immunoreactivity between

529   control (Con) and Alzheimer's disease (AD) cases in the GM and WM C).Association of AT-8 (i) and Aβ

530   (ii) immunoreactivity with Braak NFT stages across the cohort in GM and WM. Correlative analysis

531   (Spearman's r) is shown for when analysis as a single group or when separated into Con and AD groups.

532   Combined (i), Con(ii) and AD (iii), linear correlations between AT-8 and Aβ in the GM (D) and WM(E).

533   Immunoreactive shown as relative to control (Rel. Control). *=p<0.05, **=p<0.01, ***=p<0.001 and

534   ****=p<0.0001. $ denotes initial Braak NFT stage at which immunoreactivity is significantly elevated

535   from Braak 0 controls.

536

537

538

539

Figure 2. Immunohistochemical quantification of AT-8 tau and Aβ plaque burden in the frontal cortex of non-AD and AD cases. A). Example micrographs of DAB based AT-8 and 4G8 (Aβ) immunoreactivity, area of quantification following threshold application shown in red. Note the size exclusion in this parameter of intracellular 4G8 labelling to negate potential APP cross-reactivity. B). Quantification of % area coverage of AT-8 (i) and plaque (ii) immunoreactivity in control (Con) and Alzheimer's disease (AD) cases. C). Association of % area coverage of AT-8 (i) and plaques (ii) with Braak NFT stage with correlative analysis (Spearman's r) shown. Combined(i), Con(ii) and AD(iii), linear correlations between AT-8 and plaques in the GM (D). N.S = not significant, ***=p<0.001 and ****=p<0.0001. $ denotes initial Braak NFT stage at which immunoreactivity is significantly elevated from Braak 0 controls. Scale in a = 20 μm.

Figure 3. Immunohistochemical distinction between MOAB-2 labelled Aβ and APP immunoreactivity in the frontal cortex of an AD case. Example micrographs of APP (N-terminal-APP antibody) and Aβ (MOAB-2) from an AD case, in the grey (GM; a) and white matter (WM; b). Note the distinctive labelling of subcellular pools within insert (white arrows) and differential labelling of plaques (in a) Scale =20 μm.

Figure 4. Immunohistochemical quantification of intracellular Aβ and AT-8 tau in the frontal cortex of non-AD and AD cases. A). Example micrographs of AT-8 phosphorylated tau and MOAB-2 labelled intracellular Aβ in GM and WM of low (i), intermediate (ii), and high (iii) Braak stage cases. B). Quantification of intracellular Aβ (i) and AT-8 phospho-tau (ii) expressed as percentage area coverage in control (Con) and Alzheimer's disease (AD) cases. Combined(i), Con(ii) and AD(iii), spearman's correlations (r) between AT-8 and plaques in the GM (C) and WM (D). N.S = not significant, *=p<0.05, **=p<0.01, ***=p<0.001 and ****=p<0.0001. Scale =20 μm.

Figure 5. Rare instances of intracellular Aβ and tau colocalization. Example micrographs demonstrating an overlap of AT-8 and Aβ immunoreactivity within cells from a non-AD control Braak stage IV. Scale=10 μm.

18

571

572   Supplemental

573   **Supplemental table 1 – Individual case list for tissue used.** Each case was assigned an arbitrary case
574   number (Case no) and sex, age (years), post-mortem delay (PMD, hrs), Braak stage, Thal phase,
575   Consortium to Establish a Registry for Alzheimer's Disease (CERAD), the National Institute of Ageing –
576   Alzheimer's Association (NIA-AA) criteria, Lewy body (LB) Braak stage and McKeith criteria and tissue
577   use of either biochemical(Biochem) analysis, tissue microarray for plaque and AT-8 pathology (TMA)
578   and immunohistochemistry for intracellular Aβ and AT-8 (IHC) is provided.

579   **Supplemental table 2. Correlation matrix of biochemical measures of Aβ and AT-8 reactive tau**
580   **with neuropathological assessment.** Spearman correlations (r) between AT-8 and Aβ and Braak NFT
581   staging, Thal phase, CERAD, NIA-AA, Age and postmortem delay (PMD) are shown for both the grey
582   matter (GM) and white matter (WM) samples. Note for Braak stage correlations reported are
583   controlled for the influence of age. N.S = not significant, *=p<0.05, and **=p<0.01.

584   **Supplemental table 3. Correlation matrix of immunohistochemical measures of Aβ plaques and**
585   **AT-8 reactive tau with neuropathological assessment.** Spearman correlations (r) between AT-8 and
586   Aβ and Braak NFT staging, Thal phase, CERAD, NIA-AA, Age and postmortem delay (PMD) are shown.
587   Note for Braak stage correlations reported are controlled for the influence of age. N.S = not
588   significant, *=p<0.05, and **=p<0.01.

589   **Supplemental table 4. Correlation matrix of all measures of Aβ and AT-8 reactive tau across the**
590   **study** Spearman correlations (r) between AT-8 and Aβ and Braak NFT staging, Thal phase, CERAD,
591   NIA-AA, Age and post-mortem delay (PMD) are shown. Note for Braak stage correlations reported
592   are controlled for the influence of age. N.S = not significant, *=p<0.05, and **=p<0.01.

593   **Supplemental table 5. Correlation matrix of immunohistochemical measures of intracellular Aβ**
594   **and AT-8 reactive tau..** Spearman correlations (r) between AT-8 and Aβ and Braak NFT staging, Thal
595   phase, CERAD, NIA-AA, Age and postmortem delay (PMD) are shown for both the grey matter (GM)
596   and white matter (WM) samples. Note for Braak stage correlations reported are controlled for the
597   influence of age. N.S = not significant, *=p<0.05, and **=p<0.01.

598

599

600

601

602

603

604

605

606

607

References

609   1.   Hardy, J.A. and G.A. Higgins, *Alzheimer's disease: the amyloid cascade hypothesis.* Science,
610   1992. **256**(5054): p. 184-5.
611   2.   Hardy, J. and D.J. Selkoe, *The amyloid hypothesis of Alzheimer's disease: progress and*
612   *problems on the road to therapeutics.* Science, 2002. **297**(5580): p. 353-6.
613   3.   Cummings, J., et al., *Aducanumab produced a clinically meaningful benefit in association*
614   *with amyloid lowering.* Alzheimers Res Ther, 2021. **13**(1): p. 98.
615   4.   McDade, E., et al., *Lecanemab in patients with early Alzheimer's disease: detailed results on*
616   *biomarker, cognitive, and clinical effects from the randomized and open-label extension of*
617   *the phase 2 proof-of-concept study.* Alzheimers Res Ther, 2022. **14**(1): p. 191.
618   5.   van Dyck, C.H., et al., *Lecanemab in Early Alzheimer's Disease.* N Engl J Med, 2023. **388**(1): p.
619   9-21.
620   6.   Thal, D.R., et al., *Phases of A beta-deposition in the human brain and its relevance for the*
621   *development of AD.* Neurology, 2002. **58**(12): p. 1791-800.
622   7.   Mattsson, N., et al., *Staging β-Amyloid Pathology With Amyloid Positron Emission*
623   *Tomography.* JAMA Neurol, 2019. **76**(11): p. 1319-1329.
624   8.   Braak, H. and E. Braak, *Staging of Alzheimer's disease-related neurofibrillary changes.*
625   Neurobiol Aging, 1995. **16**(3): p. 271-8; discussion 278-84.
626   9.   Braak, H., et al., *Stages of the pathologic process in Alzheimer disease: age categories from 1*
627   *to 100 years.* J Neuropathol Exp Neurol, 2011. **70**(11): p. 960-9.
628   10.   Spires-Jones, T.L., J. Attems, and D.R. Thal, *Interactions of pathological proteins in*
629   *neurodegenerative diseases.* Acta Neuropathol, 2017. **134**(2): p. 187-205.
630   11.   Zhang, Y., et al., *Tauopathies: new perspectives and challenges.* Mol Neurodegener, 2022.
631   **17**(1): p. 28.
632   12.   Crary, J.F., et al., *Primary age-related tauopathy (PART): a common pathology associated*
633   *with human aging.* Acta Neuropathol, 2014. **128**(6): p. 755-66.
634   13.   DeVos, S.L., et al., *Synaptic Tau Seeding Precedes Tau Pathology in Human Alzheimer's*
635   *Disease Brain.* Front Neurosci, 2018. **12**: p. 267.
636   14.   Näslund, J., et al., *Correlation between elevated levels of amyloid beta-peptide in the brain*
637   *and cognitive decline.* Jama, 2000. **283**(12): p. 1571-7.
638   15.   Koss, D.J., et al., *Soluble pre-fibrillar tau and β-amyloid species emerge in early human*
639   *Alzheimer's disease and track disease progression and cognitive decline.* Acta Neuropathol,
640   2016. **132**(6): p. 875-895.
641   16.   Koss, D.J., et al., *Distinctive temporal profiles of detergent-soluble and -insoluble tau and Aβ*
642   *species in human Alzheimer's disease.* Brain Res, 2018. **1699**: p. 121-134.
643   17.   Horie, K., et al., *Regional correlation of biochemical measures of amyloid and tau*
644   *phosphorylation in the brain.* Acta Neuropathol Commun, 2020. **8**(1): p. 149.
645   18.   Lasagna-Reeves, C.A., et al., *Tau oligomers impair memory and induce synaptic and*
646   *mitochondrial dysfunction in wild-type mice.* Mol Neurodegener, 2011. **6**: p. 39.
647   19.   Nicole, O., et al., *Soluble amyloid beta oligomers block the learning-induced increase in*
648   *hippocampal sharp wave-ripple rate and impair spatial memory formation.* Sci Rep, 2016. **6**:
649   p. 22728.
650   20.   Bolós, M., et al., *Soluble Tau has devastating effects on the structural plasticity of*
651   *hippocampal granule neurons.* Transl Psychiatry, 2017. **7**(12): p. 1267.
652   21.   Koss, D.J., et al., *Polymeric alkylpyridinium salts permit intracellular delivery of human Tau in*
653   *rat hippocampal neurons: requirement of Tau phosphorylation for functional deficits.* Cell
654   Mol Life Sci, 2015. **72**(23): p. 4613-32.

655 22. Tomiyama, T., et al., *A mouse model of amyloid beta oligomers: their contribution to synaptic alteration, abnormal tau phosphorylation, glial activation, and neuronal loss in vivo.* J Neurosci, 2010. **30**(14): p. 4845-56.

658 23. Busche, M.A., et al., *Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease.* Proc Natl Acad Sci U S A, 2012. **109**(22): p. 8740-5.

660 24. Ben-Nejma, I.R.H., et al., *Increased soluble amyloid-beta causes early aberrant brain network hypersynchronisation in a mature-onset mouse model of amyloidosis.* Acta Neuropathol Commun, 2019. **7**(1): p. 180.

663 25. Berger, Z., et al., *Accumulation of pathological tau species and memory loss in a conditional model of tauopathy.* J Neurosci, 2007. **27**(14): p. 3650-62.

665 26. Kopeikina, K.J., et al., *Tau accumulation causes mitochondrial distribution deficits in neurons in a mouse model of tauopathy and in human Alzheimer's disease brain.* Am J Pathol, 2011. **179**(4): p. 2071-82.

668 27. Koss, D.J., et al., *Mutant Tau knock-in mice display frontotemporal dementia relevant behaviour and histopathology.* Neurobiol Dis, 2016. **91**: p. 105-23.

670 28. Jin, M., et al., *Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration.* Proc Natl Acad Sci U S A, 2011. **108**(14): p. 5819-24.

673 29. Ciccone, R., et al., *Amyloid β-Induced Upregulation of Na(v)1.6 Underlies Neuronal Hyperactivity in Tg2576 Alzheimer's Disease Mouse Model.* Sci Rep, 2019. **9**(1): p. 13592.

675 30. Selkoe, D.J. and J. Hardy, *The amyloid hypothesis of Alzheimer's disease at 25 years.* EMBO Mol Med, 2016. **8**(6): p. 595-608.

677 31. Beckman, D., et al., *Oligomeric Aβ in the monkey brain impacts synaptic integrity and induces accelerated cortical aging.* Proc Natl Acad Sci U S A, 2019. **116**(52): p. 26239-26246.

679 32. Baerends, E., et al., *Modeling the early stages of Alzheimer's disease by administering intracerebroventricular injections of human native Aβ oligomers to rats.* Acta Neuropathol Commun, 2022. **10**(1): p. 113.

682 33. Bolmont, T., et al., *Induction of tau pathology by intracerebral infusion of amyloid-beta - containing brain extract and by amyloid-beta deposition in APP x Tau transgenic mice.* Am J Pathol, 2007. **171**(6): p. 2012-20.

685 34. Aoki, M., et al., *Amyloid beta-peptide levels in laser capture microdissected cornu ammonis 1 pyramidal neurons of Alzheimer's brain.* Neuroreport, 2008. **19**(11): p. 1085-9.

687 35. Perdigão, C., et al., *Intracellular Trafficking Mechanisms of Synaptic Dysfunction in Alzheimer's Disease.* Front Cell Neurosci, 2020. **14**: p. 72.

689 36. Umeda, T., et al., *Intraneuronal amyloid β oligomers cause cell death via endoplasmic reticulum stress, endosomal/lysosomal leakage, and mitochondrial dysfunction in vivo.* J Neurosci Res, 2011. **89**(7): p. 1031-42.

692 37. Nishitsuji, K., et al., *The E693Delta mutation in amyloid precursor protein increases intracellular accumulation of amyloid beta oligomers and causes endoplasmic reticulum stress-induced apoptosis in cultured cells.* Am J Pathol, 2009. **174**(3): p. 957-69.

695 38. Montine, T.J., et al., *National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach.* Acta Neuropathol, 2012. **123**(1): p. 1-11.

698 39. Braak, H., et al., *Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry.* Acta Neuropathol, 2006. **112**(4): p. 389-404.

700 40. Hyman, B.T., et al., *National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease.* Alzheimers Dement, 2012. **8**(1): p. 1-13.

702 41. Braak, H., et al., *Staging of brain pathology related to sporadic Parkinson's disease.* Neurobiol Aging, 2003. **24**(2): p. 197-211.

704 42. McKeith, I.G., et al., *Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium.* Neurology, 2017. **89**(1): p. 88-100.

43. McKeith, I.G., et al., *Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium.* Neurology, 2005. **65**(12): p. 1863-72.

44. Walker, L., et al., *Quantitative neuropathology: an update on automated methodologies and implications for large scale cohorts.* J Neural Transm (Vienna), 2017. **124**(6): p. 671-683.

45. Iulita, M.F., et al., *Intracellular Aβ pathology and early cognitive impairments in a transgenic rat overexpressing human amyloid precursor protein: a multidimensional study.* Acta Neuropathol Commun, 2014. **2**: p. 61.

46. Youmans, K.L., et al., *Intraneuronal Aβ detection in 5xFAD mice by a new Aβ-specific antibody.* Mol Neurodegener, 2012. **7**: p. 8.

47. Arriagada, P.V., et al., *Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease.* Neurology, 1992. **42**(3 Pt 1): p. 631-9.

48. Guillozet, A.L., et al., *Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment.* Arch Neurol, 2003. **60**(5): p. 729-36.

49. Collins-Praino, L.E., et al., *Soluble amyloid beta levels are elevated in the white matter of Alzheimer's patients, independent of cortical plaque severity.* Acta Neuropathol Commun, 2014. **2**: p. 83.

50. Wu, R., et al., *Seeding-Competent Tau in Gray Matter Versus White Matter of Alzheimer's Disease Brain.* J Alzheimers Dis, 2021. **79**(4): p. 1647-1659.

51. Zheng, W.H., et al., *Amyloid beta peptide induces tau phosphorylation and loss of cholinergic neurons in rat primary septal cultures.* Neuroscience, 2002. **115**(1): p. 201-11.

52. De Felice, F.G., et al., *Alzheimer's disease-type neuronal tau hyperphosphorylation induced by A beta oligomers.* Neurobiol Aging, 2008. **29**(9): p. 1334-47.

53. Ma, Q.L., et al., *Antibodies against beta-amyloid reduce Abeta oligomers, glycogen synthase kinase-3beta activation and tau phosphorylation in vivo and in vitro.* J Neurosci Res, 2006. **83**(3): p. 374-84.

54. Zhang, H.Y., et al., *Reduction of amyloid beta by Aβ3-10-KLH vaccine also decreases tau pathology in 3×Tg-AD mice.* Brain Res Bull, 2018. **142**: p. 233-240.

55. Blennow, K., et al., *Effect of immunotherapy with bapineuzumab on cerebrospinal fluid biomarker levels in patients with mild to moderate Alzheimer disease.* Arch Neurol, 2012. **69**(8): p. 1002-10.

56. Lehmann, S., et al., *Cerebrospinal fluid A beta 1-40 peptides increase in Alzheimer's disease and are highly correlated with phospho-tau in control individuals.* Alzheimers Res Ther, 2020. **12**(1): p. 123.

57. Koo, E.H. and S.L. Squazzo, *Evidence that production and release of amyloid beta-protein involves the endocytic pathway.* J Biol Chem, 1994. **269**(26): p. 17386-9.

58. Fourriere, L. and P.A. Gleeson, *Amyloid β production along the neuronal secretory pathway: Dangerous liaisons in the Golgi?* Traffic, 2021. **22**(9): p. 319-327.

59. Tam, J.H., C. Seah, and S.H. Pasternak, *The Amyloid Precursor Protein is rapidly transported from the Golgi apparatus to the lysosome and where it is processed into beta-amyloid.* Mol Brain, 2014. **7**: p. 54.

60. Brewer, G.J., et al., *Age-Related Intraneuronal Aggregation of Amyloid-β in Endosomes, Mitochondria, Autophagosomes, and Lysosomes.* J Alzheimers Dis, 2020. **73**(1): p. 229-246.

61. Koss, D.J., et al., *RAB39B is redistributed in dementia with Lewy bodies and is sequestered within aβ plaques and Lewy bodies.* Brain Pathol, 2020.

62. Jordan, K.L., et al., *Therapeutic Targeting of Rab GTPases: Relevance for Alzheimer's Disease.* Biomedicines, 2022. **10**(5).

63. Lai, A.Y. and J. McLaurin, *Mechanisms of amyloid-Beta Peptide uptake by neurons: the role of lipid rafts and lipid raft-associated proteins.* Int J Alzheimers Dis, 2010. **2011**: p. 548380.

64. Welikovitch, L.A., et al., *Evidence of intraneuronal Aβ accumulation preceding tau pathology in the entorhinal cortex.* Acta Neuropathol, 2018. **136**(6): p. 901-917.

756 65. Gouras, G.K., et al., *Intraneuronal Abeta42 accumulation in human brain.* Am J Pathol, 2000.
757   **156**(1): p. 15-20.
758 66. Wirths, O., et al., *Intraneuronal Abeta accumulation precedes plaque formation in beta-*
759   *amyloid precursor protein and presenilin-1 double-transgenic mice.* Neurosci Lett, 2001.
760   **306**(1-2): p. 116-20.
761 67. Wegiel, J., et al., *Intraneuronal Abeta immunoreactivity is not a predictor of brain*
762   *amyloidosis-beta or neurofibrillary degeneration.* Acta Neuropathol, 2007. **113**(4): p. 389-
763   402.
764 68. Oddo, S., et al., *A dynamic relationship between intracellular and extracellular pools of*
765   *Abeta.* Am J Pathol, 2006. **168**(1): p. 184-94.
766 69. Mori, C., et al., *Intraneuronal Abeta42 accumulation in Down syndrome brain.* Amyloid,
767   2002. **9**(2): p. 88-102.
768 70. Umeda, T., et al., *Intracellular amyloid β oligomers impair organelle transport and induce*
769   *dendritic spine loss in primary neurons.* Acta Neuropathol Commun, 2015. **3**: p. 51.
770 71. Silvestro, S., A. Valeri, and E. Mazzon, *Aducanumab and Its Effects on Tau Pathology: Is This*
771   *the Turning Point of Amyloid Hypothesis?* Int J Mol Sci, 2022. **23**(4).
772 72. Clavaguera, F., et al., *Transmission and spreading of tauopathy in transgenic mouse brain.*
773   Nat Cell Biol, 2009. **11**(7): p. 909-13.
774 73. Iba, M., et al., *Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a*
775   *transgenic mouse model of Alzheimer's-like tauopathy.* J Neurosci, 2013. **33**(3): p. 1024-37.
776 74. Guo, J.L., et al., *Unique pathological tau conformers from Alzheimer's brains transmit tau*
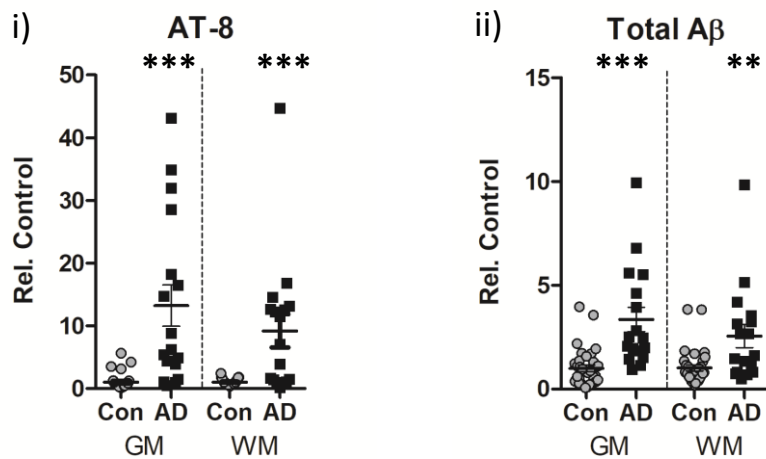777   *pathology in nontransgenic mice.* J Exp Med, 2016. **213**(12): p. 2635-2654.

778

Disease	N	Sex (% Male)	Age (years)	PMI (hrs)	NFT Braak stage	Thal Phase	CERAD	NIA-AA	LB Braak stage	McKeith Criteria
Immuno-blots										
Con	35	57.1%	47-97 80.5±2.2	16-95 45.8±3.7	0-IV 17.1% -0 17.1%- I 25.7% - II 25.7%-III 14.3%-VI	0-4 20%-0 22.9%-1 25.7%-2 22.9%-3 8.6%-4	0-2 80%-0 5.7%-1 14.3%-2	0-2 20 %- 0 65.7 % - 1 14.3 % - 2	0-3 91.4%-0 2.9%-1 5.7%-3	91.4%-No LB 8.6%-Brainstem
AD	17	35.3%	74-96 85.5±1.5	5-90 57.2±5.7	V-VI 29.4% -V 70.6% -VI	4-5 15.4%-4 84.6%-5	3 100% -3	3 100% - 3	0-3 82.3%-0 11.8%-2 5.9%-3	76.5% -No LB 17.6% -Brainstem 5.9%-Limbic
Immunohistochemistry (AT-8 and 48G plaques)										
Con	36	56.2%	55-97 81.5±2.2	16-95 47.8 ±3.9	0-IV 16.7% -0 19.4%- I 25% - II 25%-III 13.9%-VI	0-4 16.7%-0 25%-1 30.6%-2 19.4%-3 8.3%-4	0-2 77.8%-0 8.3%-1 13.9%-2	0-2 16.7%-0 69.4%-1 13.9%-2	0-3 88.1%-0 5.8%-1 6.1%-3	88.7%-No LB 8.3%-Brainstem 2.8%-Limbic
AD	20	30%	70-93 85±1.3	5-90 54.4±4.9	V-VI 25%-V 75%-VI	4-5 13.9%-4 86.1%-5	3 100%-3	3 100%-3	0-3 85%-0 10%-2 5%-3	80% -No LB 15%-Brainstem 5%-Limbic
Immunohistochemistry (AT-8 and MOAB-2 intracellular Aβ)										
Con	14	28.6%	70-97 85.6±2.4	16-95 45.1 ±7.3	0-IV 7.1% -0 14.3%- I 42.9% - II 35.7%-III	0-2 28.6%-0 35.7%-1 35.7%-2	0-1 85.7%-0 14.3%-1	0-1 35.7%-0 64.3%-1	0-3 85.7%-0 14.3%-3	78.6%-No LB 14.3%-Brainstem 7.1%-Limbic
AD	8	12.5%	78-93 85.4±1.8	29-90 57±7.5	V-VI 12.5%-V 87.5%-VI	4-5 12.5%-4 87.5%-5	3 100%-3	3 100%-3	0-3 87.5%-0 12.5%-2	75% -No LB 25%-Brainstem

Table 1. Human tissue cohort. Human cases using in tissue microarray and western blot separated by disease classification according to non-diseased controls (Con), Alzheimer's disease (AD) and dementia with Lewy bodies (DLB). Case numbers (n), sex, age, post-mortem interval (PMI), neurofibrillary tangle (NFT) Braak stage, Thal phase, Consortium to Establish a Registry for Alzheimer's Disease (CERAD), the National Institute of Ageing – Alzheimer's Association (NIA-AA) criteria, Lewy body (LB) Braak stage and McKeith criteria are provided. For age and PMI both range and mean ±SEM are provided. For numerical scores of pathology, range and percentage composition are given. For McKeith criteria, only percentage composition is given, where cases free of LBs (No LB), amygdala predominate (Amyg), limbic predominate (Limb) and neocortical predominate (Neo) are indicated. * indicates composition based on available data.

Figure 1

Figure 2

Figure 3

A) GM

APP | Aβ (MOAB-2) | APP/Aβ (MOAB-2) | APP/Aβ (MOAB-2)/DAPI

B) WM

Figure 4

A)
i) Low Braak NFT Case (Br I)
AT-8   Aβ (MOAB-2)   Merge
GM
WM

ii) intermediate Braak NFT Case (Br III)
AT-8   Aβ (MOAB-2)   Merge
GM
WM

iii) High Braak NFT Case (Br VI)
AT-8   Aβ (MOAB-2)   Merge
GM
WM

B) i) Intracellular Aβ
ii) AT-8

C) i) AT-8 vs. Intracellular Aβ- GM
Total; ****,r =0.76
Con; ***,r =0.82
AD; *, r=-0.74

ii) AT-8 vs. Intracellular Aβ- GM
Con;**,r=0.79

iii) AT-8 vs. Intracellular Aβ- GM
AD;*,r=-0.7381

D) i) AT-8 vs. Intracellular Aβ- WM
Total; ***,r =0.71
Con; **,r =0.75
AD; N.S

ii) AT-8 vs. Intracellular Aβ- WM
Con; *;r=0.75

iii) AT-8 vs. Intracellular Aβ- WM
AD = N.S

Figure 5

i)

| AT-8 | Aβ (MOAB-2) | AT-8/Aβ (MOAB-2) | AT-8/Aβ (MOAB-2)/DAPI |

ii)