Searching for novel cerebrospinal fluid biomarkers of tau pathology in frontotemporal dementia: an elusive quest

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INTRODUCTION

Frontotemporal dementia (FTD) is a common form of early-onset dementia, but it is pathologically heterogeneous, which precludes accurate diagnosis during life of the underlying molecular cause.1 The majority of patients with FTD have either tau or TDP-43 inclusions at post mortem, but at present there are no biomarkers that can reliably separate these groups from each other or from healthy controls. Currently available cerebrospinal fluid (CSF) measures of tau do not seem to represent the burden of cerebral tau pathology and are variably affected in different forms of FTD. Furthermore, they can be abnormal in other proteinopathies.2–5 However, studies indicate that the tau protein can be cleaved into multiple different fragments, which are actively secreted from cells and can therefore potentially be identified in CSF.6–7 In this study, we assessed the potential of novel CSF measures of different tau species as candidate biomarkers for FTD.

METHODS

Participants

86 consecutively recruited participants with available CSF from the University College London FTD cohort studies were included in the study: 66 patients and 20 healthy cognitively normal controls. The 66 patients met consensus diagnostic criteria for either behavioural variant FTD (bvFTD) (21, of whom one patient had associated motor neuron disease) or primary progressive aphasia (PPA) (45). In the PPA cohort, 11 had semantic variant, 16 had non-fluent variant (of whom two patients had associated progressive supranuclear palsy), 15 had logopenic variant and 3 did not meet criteria for any of the three variants, named PPA-not otherwise specified (PPA-NOS).9 All patients were screened using sandwich ELISAs (INNOTEST; Fujirebio Europe N.V., Gent, Belgium) following manufacturers’ instructions.

Measurement of CSF markers

CSF was collected, processed and stored at −80°C according to standardised procedures.10 11 Initially, the concentrations of the currently available markers of CSF T-tau, P-tau(181) and Aβ42 were determined using sandwich ELISAs (INNOTEST; Fujirebio Europe N.V., Gent, Belgium) following manufacturer’s instructions.

We then performed five further ELISAs, one previously reported to identify non-phosphorylated...
Figure 1 (Continued)
forms of tau (performed as in Lewczuk et al12), and four novel assays (figure 1A; online supplementary material):

- **Tau N-123 amino acid (aa):** Plates were coated and incubated overnight at +4°C with in-house antibody anti-tau 123. Titrated calibrators (123 recombinant tau fragment) and sample were co-incubated with biotinylated detection antibody Tau 12 (Nordic Biosite). For detection, enhanced streptavidin–horseradish peroxidase (HRP) complex was used.

- **Tau N-224 amino acid (aa):** Magnetic beads (Quanterix, Lexington, Massachusetts, USA) were conjugated with the capture antibody anti-Tau 224 according to bead supplier’s conjugation protocol. Prior to each run, Tau 224 recombinant protein was serially diluted and used.
biont-labelled antibody Tau 12 (Nordic Biosite) was used for detection.

- Tau X-368aa: Magnetic beads (Quanterix) were conjugated with capture antibody anti-Tau368, Tau 1–368 recombinant protein was serially diluted and used as calibrator. As detection antibody, biotin-labelled K9JA (Sigma) was used.

- Tau N-mid-region: Tau12 (Nordic Biosite, binding region aa9–18) was used as coating antibody and, as for detection, a combination of biotinylated HT7 (Thermo Scientific, aa159–163) and BT2 (Thermo Scientific, aa194–198). For detection, enhanced streptavidin–HRP complex was used. Full-length recombinant Tau 441 2N4R (rPeptide) was used as calibrator.

**Statistical analysis**
Concentrations of tau species were compared between groups using a linear regression model in STATA V.14 (StataCorp, College Station, Texas, USA) with 95% bias-corrected bootstrapped CIs with 1000 repetitions. There was no difference in age and gender between controls and each of the disease groups (all comparisons >0.05; Kruskal-Wallis test, age; Fisher’s exact test, gender). The optimal cut-off point for each tau marker to differentiate tau from TDP-43 pathology was identified by selecting the concentration that produced the highest Youden index (J=sensitivity+specificity–1) using GraphPad Prism V.7 (GraphPad Software, San Diego, California, USA). The area under the curve (AUC) was calculated for each comparison. Spearman’s correlation coefficient (p) was used to investigate the association between tau species.

**RESULTS**
Comparison of those with likely AD versus FTLD pathology (figure 1B, table 1, online supplementary table)

Some patients with bvFTD or PPA (particularly the logopenic variant) may have underlying Alzheimer’s disease (AD) pathology rather than frontotemporal lobar degeneration (FTLD) pathology. The initial analysis therefore aimed to compare these groups with controls. We used the Duits criteria\(^{13}\) to identify those patients who were likely to have underlying AD pathology (i.e. CSF concentrations of A\(_{β}\)<550 pg/mL). This atypical AD group of 21 patients included 14 with logopenic variant PPA, 3 with non-fluent variant PPA, 1 with semantic variant PPA, 1 with PPA-NOS and 2 with bvFTD. Forty-five patients had A\(_{β}\)>550 pg/mL and therefore were included in the FTLD group figure 2.

The mean (SD) T-tau and P-tau concentrations were significantly higher in the AD group (722.7 (529.1) pg/mL; 72.1 (42.3) pg/mL) compared with healthy controls (326.6 (90.8) pg/mL; 51.1 (11.9) pg/mL) and the FTLD group (464.7 (344.1) pg/mL; 51.3 (24.1) pg/mL).

The AD group also showed significantly higher concentrations than controls for tau N-mid-region (AD, 294.7 (179.2) pg/mL; controls, 153.4 (63.8) pg/mL), tau N-224 (10.8 (8.2) pg/mL; 6.0 (3.4) pg/mL) and non-phosphorylated tau (77.2 (53.4) pg/mL; 39.4 (17.6) pg/mL). However, no significant differences were seen for tau N-123 (77.4 (77.4) pg/mL; 85.8 (115.7) pg/mL) or tau X-368 (16.3 (6.3) pg/mL; 16.0 (4.1) pg/mL).

The FTLD group showed significantly higher concentrations than controls for N-mid-region (216.7 (132.6) pg/mL), tau N-224 (9.3 (8.2) pg/mL) and non-phosphorylated tau (57.0 (35.7) pg/mL) but not for other novel measures.

None of the novel measures showed a significant difference between the AD and FTLD group.

**Comparison of those with likely FTLD-tau versus FTLD-TDP-43 pathology (figures 1C and D; table 1, online supplementary table)**

Individuals in the FTLD group were then grouped based on their likely underlying pathology into an FTLD-tau group (containing MAPT mutation carriers, those with a secondary clinical diagnosis of progressive supranuclear palsy, and one patient with bvFTD who had subsequently come to post mortem and was found to have corticobasal degeneration; n=7) and an FTLD-TDP-43 group (containing GRN and C9orf72 mutation carriers, those with a primary clinical

**Table 1**
Demographics and tau CSF marker concentrations for healthy controls, groups split by Duits criteria for likely AD pathology (vs FTLD pathology) and groups split by likely FTLD-tau and FTLD-TDP-43 pathology

<table>
<thead>
<tr>
<th>N</th>
<th>Male gender (% group)</th>
<th>Age at CSF (years) (Mean (SD))</th>
<th>T-tau pg/mL (Mean (SD))</th>
<th>P-tau pg/mL (Mean (SD))</th>
<th>Tau N-123 pg/mL (Mean (SD))</th>
<th>Tau N-mid-region pg/mL (Mean (SD))</th>
<th>Tau N-224 pg/mL (Mean (SD))</th>
<th>Non-phosphorylated tau pg/mL (Mean (SD))</th>
<th>Tau X-368 pg/mL (Mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>10 (50.0)</td>
<td>64.2 (6.9)</td>
<td>326.6 (90.8)</td>
<td>51.1 (11.9)</td>
<td>85.8 (115.7)</td>
<td>153.4 (63.8)</td>
<td>6.0 (3.4)</td>
<td>39.4 (17.6)</td>
<td>16.0 (4.1)</td>
</tr>
<tr>
<td>21</td>
<td>11 (52.3)</td>
<td>65.8 (6.1)</td>
<td>722.7 (529.1)</td>
<td>72.1 (42.3)</td>
<td>77.4 (77.4)</td>
<td>294.7 (179.2)</td>
<td>10.8 (8.2)</td>
<td>77.2 (53.4)</td>
<td>16.3 (6.3)</td>
</tr>
<tr>
<td>45</td>
<td>34 (75.5)</td>
<td>64.1 (6.6)</td>
<td>464.7 (344.1)</td>
<td>51.3 (24.1)</td>
<td>74.0 (82.5)</td>
<td>216.7 (132.6)</td>
<td>9.3 (8.2)</td>
<td>57.0 (35.7)</td>
<td>15.2 (4.8)</td>
</tr>
<tr>
<td>7</td>
<td>5 (71.4)</td>
<td>64.8 (8.8)</td>
<td>444.4 (146.4)</td>
<td>53.5 (13.9)</td>
<td>54.9 (53.5)</td>
<td>215.4 (71.4)</td>
<td>13.9 (13.1)</td>
<td>51.4 (20.7)</td>
<td>15.1 (2.9)</td>
</tr>
<tr>
<td>18</td>
<td>13 (72.2)</td>
<td>62.3 (5.9)</td>
<td>457.1 (224.6)</td>
<td>47.2 (16.5)</td>
<td>70.6 (87.0)</td>
<td>235.1 (123.3)</td>
<td>8.5 (4.7)</td>
<td>62.5 (32.9)</td>
<td>15.6 (4.1)</td>
</tr>
</tbody>
</table>

AD, Alzheimer’s disease; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration.

diagnosis of semantic variant PPA or a secondary diagnosis of motor neuron disease; n=18) (figure 2).

CSF T-tau concentrations were significantly higher in the FTLD-TDP-43 group (457.1 (224.6) pg/mL) compared with controls, and also in the FTLD-tau group (444.4 (146.4) pg/mL) compared with controls.

Tau N-mid-region (235.1 (123.3) pg/mL), tau N-224 (8.5 (4.7) pg/mL) and non-phosphorylated tau (62.5 (32.9) pg/mL) also showed higher concentrations in the FTLD-TDP-43 group compared with healthy controls, while only concentrations of tau N-mid-region (215.4 (71.4) pg/mL) and tau N-224 (13.9 (13.1) pg/mL) were significantly higher in the FTLD-tau group compared with controls. No significant differences were seen for tau N-123 and tau X-368.

None of the measures showed a significant difference between the FTLD-TDP-43 and FTLD-tau groups.

We performed a subanalysis normalising tau markers for T-tau, based on previous literature which has shown an improved differentiation of tau and TDP-43 pathology using the ratio of P-tau₁₈₁ to T-tau. The P-tau₁₈₁/T-tau ratio was significantly lower for both the FTLD-TDP-43 group (mean (SD) 0.113 (0.032)) and the FTLD-tau group (0.126 (0.033)) compared with controls (0.160 (0.027)), but there was no significant difference between the FTLD-tau and FTLD-TDP-43 groups. Receiver operating characteristic (ROC) curve analysis measuring the ability of P-tau₁₈₁/T-tau to differentiate probable FTLD-tau from FTLD-TDP-43 showed a sensitivity of 61.1% and specificity of 85.7% with a cut-off point of <0.109 and an AUC 0.63.

Of the novel tau species, both tau X-368 and tau N-224 had a significantly different ratio in the FTLD-tau group (mean (SD): 0.036 (0.010); 0.035 (0.037)) compared with controls (0.050 (0.009); 0.013 (0.009)). For tau X-368, the ratio was also lower in the FTLD-TDP-43 group (0.039 (0.013)) compared with controls, but there was no difference between the FTLD-tau and FTLD-TDP-43 groups. For tau N-224, there was also a significantly higher ratio for FTLD-tau compared with the FTLD-TDP-43 group (0.019 (0.010)). However,
sensitivity and specificity of tau N-224 ratio to differentiate between likely FTLD-tau and FTLD-TDP-43 groups was only 61.1% and 57.1% with a cut-off point of <0.019 (AUC of 0.63). No significant differences were shown in the other novel tau measures.

Correlations of tau CSF biomarkers
All CSF tau markers were significantly correlated with each other (figure 1E). However, the strongest correlations were for T-tau with P-tau (t181) (r=0.87) and tau N-mid-region (r=0.84), and for tau N-mid-region with tau X-368 (r=0.86) and non-phosphorylated tau (r=0.85). Although significant, the correlations of Tau N-123 with the other tau species were fairly weak.

DISCUSSION
In this study, we investigated the potential of novel CSF tau measures as biomarkers of tau pathology in FTD. However, no significant differences were seen between those with likely underlying AD pathology and FTLD pathology, or between those with likely FTLD-tau and FTLD-TDP-43 pathology.

Tau N-224 was one of only two markers higher in the FTLD-tau group compared with controls, and when normalised for total-tau, showed a significant difference between FTLD-tau and FTLD-TDP-43, but separated the groups with only poor sensitivity and specificity of <65% (AUC of 0.63). A similar sensitivity (61.1%) and higher specificity (85.7%) was found for the P-tau (t181)/T-tau ratio at a cut-off point of <0.109 (AUC of 0.63), a marker previously described by other groups: Hu et al4 found a sensitivity of 82% and specificity of 62% for the comparison of FTLD-tau and FTLD-TDP-43 with a cut-off point of <0.372 (AUC of 0.73), while Borroni et al5 found 83% and 64% with a ratio of <0.136 (AUC of 0.87), and Meeter et al6, 67% and 76% with a cut-off point of <0.121 (AUC of 0.73). However, such a sensitivity and specificity would have limited clinical use as it would still result in considerable overlap between groups.

Although the fragments we have measured do not show a diagnostic accuracy that is superior to the existing tau biomarkers, we find different patterns in the concentrations of the fragments between the pathological groups. This finding is in concordance with other studies which suggest that tau may be differentially processed and secreted in a regulated manner.14 We hypothesise that specific tau fragments may be generated and secreted in different tauopathies, and here we provide evidence that three tau fragments are significantly increased in CSF in FTD compared with controls. It is likely that there are other fragments of tau, not analysed in this study, that are more specific to FTD, and further work is required to identify these.

This study has a number of limitations. The majority of the patients did not have pathological confirmation of the cause of their illness, and in addition, although there is a relatively large number of cases for a study of a rare disorder like FTD, the individual numbers are small in each subgroup. There are also potentially limitations in the assay sensitivities for the novel tau fragments such that improvement in these may lead to a clearer difference between cases and controls that is not currently apparent.

In conclusion, while a number of these novel tau species show significantly higher concentrations in those with underlying AD pathology, they do not show any added benefit above current tau biomarkers and are not useful as biomarkers of tau pathology in FTD. Further work in the development of biomarkers of tau and TDP-43 in FTD is needed, particularly in light of potential disease-modifying tau therapies currently entering clinical trials.

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Contributors JDR and MSF designed the study. JDR, MSF, CC, NE, PL and KH analysed the data. JDR and MSF wrote the initial manuscript. AK, RPW, IOCWW, CH, AJH, KY, JK, NCF, JMS, JW, HZ and KB helped with data collection, analysis and review of the manuscript.

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