Title: Untangling the tau microtubule-binding region

Running title: Tau microtubule-binding region biomarkers

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Human tau is produced as six isoforms, mainly by neurons, and subject to a huge range of post-translational modifications, as well as fragmentation by a variety of proteases, likely determining aggregation and localisation potential (Quinn *et al.*, 2018). In general, all tau functions are regulated through rigorous phosphorylation and dephosphorylation mechanisms on serine (n=45), threonine (n=35) and tyrosine (n=5) residues.

In tauopathies (neurodegenerative conditions involving tau), tau protein is typically found to accumulate within neurons (and to a much lesser extent in astrocytes and oligodendrocytes), and is released into the brain interstitial fluid as part of physiological processes and upon cell death. Brain interstitial fluid is filtered through the tight junctions of ventricular endothelial cells to become CSF, taking soluble forms of tau with it. CSF circulates through the subarachnoid space and diffuses into the venous blood, or back into brain interstitial fluid. Thus, alterations of tau concentrations in biological fluids have the potential to reflect the pathological state of brain tissue – a disease biomarker. In Alzheimer's disease (AD), but not in all tauopathies, concentrations of tau in brain interstitial fluid, cerebrospinal fluid (CSF) and blood are reliably increased early in the disease course.

Tau has seen increasing utility as a biomarker of AD, since the discovery that it forms intra-neuronal neurofibrillary tangles – one of the defining features of AD pathology. Early work using mass spectrometry was key to identifying certain phosphorylation sites on tau that are enriched in AD. CSF immunoassay of phosphorylated tau (P-tau) has become a useful complement to indiscriminate measurement of tau fragments (T-tau) and Aβ42/Aβ40 ratio as fluid biomarkers in the clinical diagnostic process. Recent improvements in assay sensitivity have begun to show diagnostic promise for measurements of P-tau$_{181,}$ P-tau$_{231}$, and P-tau$_{217}$ (though not T-tau) in blood plasma (Zetterberg and Blennow, 2020). In parallel with the development of fluid biomarkers, imaging biomarkers have evolved from seminal histochemisty work to positron emission tomography (PET) of tau deposition in the living brain. Tau PET ligands bind to aggregated tau enabling detection of early-stage pathology with high sensitivity and specificity in discriminating AD and non-AD neurodegenerative diseases, and correlate well with dynamic change in later stages pathology (e.g., brain atrophy) and cognitive function decline (e.g., MMSE score).

However, a crucial issue is that soluble tau, present in CSF and blood, is structurally and biologically different from intracellular and aggregated tau, causing a disjunction in the interpretation of one set of data versus the other. Where tau PET tracers, such as flortaucipir, and CSF T-tau and P-tau immunoassays have been tested in parallel (Mattsson *et al.*, 2017), concordance between PET and immunoassay is generally high for identifying symptomatic-stage AD versus non-AD, although this is dependent on the brain regions imaged. Additionally, CSF P-tau and flortaucipir PET show complementary ability to distinguish AD from non-AD dementia. Despite this, correspondence

between fluid and imaging biomarkers are modest at preclinical stages (CSF T-tau and P-tau are more sensitive to the earliest pathological changes), whilst in late-stage AD they diverge greatly (tau PET tracks disease progression well whilst CSF tau does not). Finally, correlations of CSF tau with amyloid PET positivity in AD suggest a soluble tau response to amyloid plaques before significant tau tangle formation can be detected by PET (Sato *et al.*, 2018). As our understanding of the role of tau in damage to the brain grows, and as tau has become a therapeutic target in clinical trials, the need to close the gap between tissue pathology and fluid matrix has become pressing.

The study by Horie *et al.*, represents one of the more comprehensive investigations of human tau fragmentation in biological fluid to date. In line with previous work, the authors used immunoprecipitation to isolate tau fragments (primarily containing the N-terminal and mid-domain regions) from samples and measured the spectrum by mass spectrometry. The authors also refined a technique for chemical extraction of tau in complement to immunoprecipitation, enabling the extraction of tau fragments regardless of conformation and sequence, overcoming traditional limitations of antibody-based extraction. This technique enabled measurement of tau forms truncated within the microtubule-binding region (MTBR-tau) and C-terminus, as well as the N-terminal and mid domains. It should be noted that the MTBR encompasses the repeat domains that define the major isoforms of tau (Figure 1).

Horie *et al.* employed a layered experiment design. First, a small-scale pilot study was conducted using post-mortem brains from individuals with and without AD, identifying tau fragments containing specific microtubule-binding regions (MTBR-tau-231, 243, 299, and 345) as enriched in AD. Current antibody-based methods are not sufficient to detect MTBR-tau fragments, indeed these fragments had also eluded the author's previous attempts at chemical isolation (Barthélemy *et al.*, 2019). The initial experiment was followed up with a larger study of 20 brains, which confirmed and expanded the initial findings by identifying additional AD-associated fragments (Figure 1). The repeat domains of tau fragments (located within the MTBR region) are known to be vital to its prion-like ability to template aggregates (Sanders *et al.*, 2014), and antibodies against tau MTBR greatly impede tau aggregation under laboratory conditions (Kontsekova *et al.*, 2014). The MTBR truncations identified by Horie *et al.* are clear candidates for the forms which may propagate tauopathy extracellularly.

Horie *et al.* then investigated whether this AD-associated tau fragmentation pattern could be detected in the CSF of 100 individuals, a question with high clinical relevance. Excitingly, it emerged that some of the MTBR-tau species identified in brain showed concentration patterns that corresponded to disease stage. Truncated MTBR-tau-243 had the greatest potential as a biomarker of disease progression, and, importantly, correlated well with tau PET. This finding stands out as

having the potential to bridge the disjunction between fluid and imaging tau biomarkers as MTBR-tau-243 appears to reflect ongoing changes in diseased tissue.

Finally, the authors analysed CSF from a smaller group of participants from whom CSF had been collected longitudinally. They found that some of the MTBR-tau species they had identified increased over time in AD, but not controls, whilst MTBR-tau-243 also increased in controls to a lesser degree. Previous work has shown longitudinal increases in CSF T-tau and P-tau$_{181}$ (Llibre-Guerra *et al.*, 2019) and plasma P-tau$_{181}$ concentration (Janelidze *et al.*, 2020) can act as early predictors of AD, whilst studies in late stage AD have shown that CSF P-tau$_{181}$ concentration declines (Seppälä *et al.*, 2011). This could be due to accumulation in the brain as seen with Aβ42, as a response to changes in the activity of soluble Aβ as it is increasingly sequestered, or as a reflection of frank neuronal loss (fewer neurons able to make and secrete tau). The fact that some forms of MTBR-tau continue to increase over the disease course might indicate a level of independence from Aβ pathobiology and correspond to other important neurodegenerative/actively toxic processes that could be therapeutic targets for those with established AD.

It is now suspected that specific truncated and modified tau isoforms are produced in response to Aβ pathology (Sato *et al.*, 2018). This has important implications for currently available tau fluid biomarkers, which could be said to be at least partly 'confounded' by Aβ-stimulated neuronal secretion, creating a circularity whereby secreted AD-specific tau becomes a biomarker of Aβ pathology. The finding that certain forms of MTBR-tau are altered in both AD post-mortem brain tissue and CSF, and that CSF MTBR-tau-243 correlates with tau PET, as well as potentially tracking dynamic changes in disease stage over time, may open a route to entirely new tau biomarkers that better reflect tau pathology in the AD brain. There are three obvious questions for future work to address: Do AD altered MTBR-tau concentrations extend to blood? What are the mechanisms by which MTBR is produced, accumulates, and might have toxic effect? And finally, are other post-translational modifications on truncated tau relevant to explore in this space? The race is on to find the answers.

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Figure 1: Showing the regional domains of the generic tau protein. Phosphorylation sites known to be hyperphosphorylated in AD, as well as other sites of post-translational modification, are demarked above the protein model. Residues identified by Horie et al. as associated with AD in brain homogenate and CSF are indicated below the protein model.