Post-Mortem validation of *in vivo* 18kDa Translocator Protein (TSPO) PET as a microglial biomarker

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Abstract

Neuroinflammation is a feature of many neurodegenerative diseases, and is quantified *in vivo* by PET imaging with radioligands for the translocator protein (TSPO, e.g. [^{11}C]-PK11195). TSPO radioligand binding correlates with clinical severity and predicts clinical progression. However, the cellular substrate of altered TSPO binding is controversial and requires neuropathological validation.

We used progressive supranuclear palsy (PSP) as a demonstrator condition, to test the hypothesis that [^{11}C]-PK11195 PET reflects microglial changes. We included people with PSP-Richardson's syndrome who had undergone [^{11}C]-PK11195 PET in life (n=8). In *post-mortem* brain tissue from the same participants, we characterised cell-type specific TSPO expression and quantified microgliosis in eight cortical and eleven subcortical regions.

Double-immunofluorescence labelling for TSPO and cell markers showed TSPO expression in microglia, astrocytes, and endothelial cells. Microglial (and not astrocytic) TSPO levels were higher in donors with PSP compared to controls (n=3), and correlated with changes in microglial density. There was a significant positive correlation between regional [^{11}C]-PK11195 binding potential *ante-mortem* and the density of *post-mortem* CD68+ phagocytic microglia, as well as microglial TSPO levels.

We conclude that *in vivo* disease-related changes in [^{11}C]-PK11195 binding is largely driven by microglia and can be interpreted as a biomarker of microglia-mediated neuroinflammation in tauopathies.

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Introduction

Neuroinflammation has emerged as an important pathological feature of multiple neurodegenerative diseases, alongside accumulation of misfolded protein aggregates, synapse loss and neuronal death[1]. Microglia have gained particular interest as a dominant part of the central neuroinflammatory response[2]. In tauopathies such as progressive supranuclear palsy (PSP), *post-mortem* evidence has associated microglial burden to neurodegeneration and tau pathology[3]. In tau models, microglia respond to tau by releasing inflammatory mediators and attempting to clear aggregates, while prolonged exposure can lead to a dysregulated response, exacerbating tau spread[4]. Given the potential for prognostication and therapeutic targeting of inflammation, biomarkers that can quantify and localise the inflammatory response *in vivo* are of high importance[5].

Several measures of neuroinflammation have emerged using PET imaging, with radioligands that bind to the 18kDa translocator protein (TSPO), such as [^{11}C]-PK11195[5]. TSPO PET has been used to quantify and localise neuroinflammatory changes in the brain of people with neurodegenerative diseases, with increased TSPO radioligand binding reported across multiple diseases, including PSP[5–11]. The regional distribution of increased TSPO radioligand binding is disease specific (for example, high in pallidum, midbrain, and frontal cortex in PSP[10]), relates to clinical severity, and predicts clinical progression in patients with PSP Richardson's syndrome[9], frontotemporal dementia[12] and amnestic Alzheimer's disease (AD)[13]. Independent studies using different TSPO PET tracers in PSP patients have found similar patterns of increased TSPO signal, inflammation progression, and association with different markers of pathology[6–10,14].

There remain controversies in the interpretation of TSPO PET[15]. The rationale of TSPO radioligands as biomarkers for neuroinflammation is based largely on autoradiography, animal model and cell culture studies. Early studies correlated TSPO autoradiography to microglial staining in neurotoxin-treated tissue and preclinical mouse models of AD[16,17], which was later corroborated by antibody-based primate and mouse studies[18,19]. However, it has been challenged whether TSPO reflects microglia specifically[15], and whether changes in density or reactivity are responsible for this[20]. Microglia do not consistently upregulate TSPO expression in response to inflammatory stimuli[20,21]. Moreover, in multiple sclerosis, increased TSPO expression has been observed in endothelium and astrocytes[22]. This prompts the question as to which cell types contribute to the disease-driven changes observed by TSPO PET imaging.

This study had two principal aims: (i) to test the hypothesis that TSPO elevation in tauopathies is microglial-specific, rather than driven by astrocytes, and (ii) to test the hypothesis that *ante-mortem* TSPO PET imaging correlates with regional and individual *post-mortem* differences in microglia. We used PSP as a demonstrator tauopathy because of its high clinicopathological correlation, prognostic relevance of increased TSPO radioligand binding, and short disease course with a short timeframe between PET and death. Confirmation of these hypotheses would support the interpretation of TSPO PET (specifically [^{11}C]-PK11195) as a microglial neuroinflammatory biomarker in primary tauopathies.

Materials and methods

Eight people with PSP (*ante-mortem* diagnosis PSP-Richardson's syndrome) who underwent [^{11}C]-PK11195 PET during life[8,10] donated their brain to the Cambridge Brain Bank. Neuropathological diagnosis of PSP was confirmed in all cases. A control group consisted of three age-matched neurologically healthy individuals with mild age-related pathology. Demographics are summarised in Table 1.

Patients underwent [^{11}C]-PK11195 PET using dynamic imaging for 75 minutes on a GE Advance or GE Discovery 690 PET/CT. Non-displaceable binding potential (BP_{ND}) was calculated in cortical (Brodmann areas) and subcortical (modified Hammersmith atlas) regions. Supervised cluster analysis was used to determine the reference tissue time-activity curve and BP_{ND} values were calculated with a simplified reference tissue model.

Double-immunofluorescence labelling in formalin-fixed paraffin-embedded *post-mortem* brain tissue sections visualised TSPO in astrocytes (GFAP), microglia (IBA1) and endothelium (CD31) in posterior frontal lobe tissue (Brodmann area 6). Leica© SPE Confocal Microscopy provided high magnification images and z-stacks. DAB-based immunohistochemistry identified CD68 in eight cortical and eleven subcortical areas. Whole-slide images were acquired with an Aperio AT2 whole-slide scanner (Leica) for immunohistochemistry and a Zeiss Axioscan Z1 Slidescanner for immunofluorescence. QuPath quantified CD68+ staining via pixel-classification in grey and white matter. Area fraction and co-localisation analysis of IBA1/TSPO and GFAP/TSPO slides were performed using a colour-thresholding pipeline in ImageJ. Area fraction was defined as the percentage of marker-positive stained area relative to the total tissue area analysed. TSPO area fraction per microglia was calculated by exporting

individual cells to ImageJ, converting them to RGB files with individual channels, and utilising the same colour thresholding pipeline (Figure 2F).

Non-parametric testing was applied to account for sample size constraints. Mann-Whitney tests compared control and PSP datasets. The Kruskal-Wallis rank sum and Dunn's post-hoc test assessed grey/white matter comparisons for IBA1-TSPO and GFAP-TSPO area fractions. A linear mixed-effects model tested the association between *in vivo* [^{11}C]-PK11195 binding potential (BP_{ND}) and CD68+ microglia quantification across regions. Spearman's correlation analysed the association between (i) microglial TSPO area fraction and total microglia, and (ii) *in vivo* [^{11}C]-PK11195 BP_{ND} and TSPO-IBA1 and TSPO-GFAP area fractions in frontal lobe.

Please refer to Supplementary Material 1 for detailed Materials and Methods.

Results

TSPO showed a multicellular expression profile in *post-mortem* brain tissue

To investigate the cellular substrate of [^{11}C]-PK11195 binding, we characterised the TSPO expression profile in human *post-mortem* tissue. Immunohistochemical staining revealed ubiquitous expression of TSPO across white and grey matter of the frontal lobe with a strong expression in the vasculature (Figure 1A). In addition, non-vascular cellular expression was observed, with staining morphology suggestive of a glial origin.

Cell-type specific expression of TSPO was determined by immunofluorescence labelling for TSPO together with cell-type markers (IBA1 for microglia, GFAP for astrocytes and CD31 for endothelial cells). IBA1-TSPO co-staining revealed TSPO expression in a substantial proportion of the IBA1+ microglia, showing a punctate TSPO pattern in microglial soma and processes across white and grey matter (Figure 1B-D). CD31-TSPO co-staining showed abundant expression of TSPO in endothelial cells, and this was observed across capillaries, arterioles, venules, arteries and veins (Figure 1E). GFAP-TSPO co-staining demonstrated sparse astrocytic TSPO expression, with only rare GFAP+ astrocytes showing co-localisation with TSPO staining (Figure 1F-H).

Quantification of cell-type specific expression revealed a microglia-specific increase of TSPO in PSP

Next, we investigated which cell type drives the increase in TSPO radioligand binding in PSP. Since PSP lacks a significant vascular contribution and the cellular substrate of the TSPO signal is mainly contested between the glial cell types[15], we focused our analysis on astrocytic and microglial expression. We quantified the area of IBA1-TSPO and GFAP-TSPO co-localisation (reflective of microglial and astrocytic TSPO levels respectively) in posterior frontal lobe (BA6) tissue from neuropathologically confirmed PSP donors who had undergone [^{11}C]-PK11195 PET during life[8,10], and control donors.

Quantification of TSPO confirmed an increase in total TSPO levels in PSP brain tissue as compared to controls (Figure 2A). There was a significant increase in the microglial TSPO levels in PSP versus controls (Figure 2B). Comparison of microglial TSPO levels in white and grey matter revealed an increase in both compartments in PSP versus controls, although this was more pronounced and statistically significant in white matter (Figure 2C). Astrocytic TSPO levels did not differ significantly between PSP and control tissue, in frontal lobe as a whole (Figure 2D) or in white and grey matter compartments (Figure 2E). This data indicate that the disease-related TSPO radioligand binding in PSP is predominantly driven by microglia, rather than astrocytes, in particular microglia in white matter.

To further investigate the basis of increased microglial TSPO levels in PSP tissue, we assessed TSPO area per microglial cell as a measure of cellular expression. There was no difference in the TSPO levels per cell between PSP and controls (Figure 2F-G). Furthermore, we found a positive correlation between microglial TSPO levels and microglial density (Figure 2H), with microglial density showing a (non-significant) 1.9 fold increase in PSP versus controls (Supplementary Figure 1). These data suggests that a higher density of microglia, rather than TSPO expression per microglial cell, underlies the elevated microglial TSPO levels.

Post-mortem microgliosis and microglial TSPO expression correlated with *ante-mortem* TSPO radioligand binding

To confirm that TSPO radioligand binding determined with PET in PSP reflects microglial reactivity, we assessed the regional association between histologically-determined microgliosis in *post-mortem* brain tissue and [^{11}C]-PK11195 binding potential (BP_{ND}) from the same PSP

donors during life[8,10]. "Phagocytic" microglia were quantified using CD68 immunohistochemistry across 8 cortical and 11 subcortical regions. Area fractions of CD68+ microglia were the highest across the cortical white matter regions and subcortical regions (Figure 3A). [^{11}C]-PK11195 BP_{ND} was calculated for each individual donor for each region (Figure 3B). The model comparison of three linear mixed effects models identified one with a fixed term for the effect of [^{11}C]-PK11195 BP_{ND} on CD68 area fraction, and random intercept for individual patients (*Δchi-square (1)* = 4.47, $p = 0.0345$), but not random slope (*Δchi-square (1)* = 1.89, $p = 0.39$). The optimal model confirmed the significant positive association between *in vivo* TSPO radioligand binding and the area fraction of *post-mortem* CD68-positive 'phagocytic' microglia (*Est* = 0.042, $t = 3.43$ $p = 0.00076$; Figure 3C). The association remained significant (*Est* = 0.042, $t = 3.43$ $p = 0.00077$) with the addition of covariates for the interval from PET scanning to death (*Est* = 0.0009, $t = 1.41$ $p = 0.219$) and PSP pathology stage (*Est* = -0.0035, $t = -0.47$ $p = 0.656$)[23].

There was a significant positive association between *in vivo* TSPO radioligand binding and microglial TSPO levels in the frontal lobe (*Spearman's* $\rho = 0.839$, $p = 0.001$; Figure 3D), whilst an association with astrocytic TSPO levels was not significant (*Spearman's* $\rho = 0.510$, $p = 0.09$; Figure 3E).

Discussion

This is the first study to show TSPO PET-to-Pathology correlations using *post-mortem* brain tissue from participants in *ante-mortem* [^{11}C]-PK11195 PET studies. Our key findings are that: (i) despite TSPO's multicellular expression, its increased levels in disease was microglial (over astrocytic) in origin, which correlated with microglial density, and (ii) TSPO radioligand binding during life was significantly associated with *post-mortem* CD68+ microglia and microglial (but not astrocytic) TSPO levels. These findings indicate that TSPO PET in a primary tauopathy can be interpreted as a microglial-specific neuroinflammatory biomarker, supporting interpretations of previous PET studies in PSP reporting increased TSPO radioligand binding[6–10,14].

TSPO expression in multiple brain cell types was confirmed, consistent with prior studies[22,24,25]. However, our cell-type specific TSPO expression studies identified microglia as the key driver of increased TSPO levels in disease. Our finding that microglial density rather

than elevated cellular TSPO expression contributes to this increase is consistent with recent work in the field[20]. In brain donors who underwent [^{11}C]-PK11195 PET during life, we compared *post-mortem* quantifications of microglia and cell-type specific TSPO levels with their corresponding *in vivo* TSPO radioligand binding. The TSPO PET binding *in vivo* correlated with CD68-positive phagocytic microglia *post-mortem*, and did so across multiple cortical and subcortical brain regions. In the frontal cortex, we confirmed the association of *in vivo* TSPO radioligand binding with microglial TSPO levels *post-mortem*, while no such association was found with astrocytic TSPO. Taken together, these findings indicate that while TSPO radioligand binding likely reflects contributions from various TSPO-expressing cell types, its increase in PSP is primarly of microglial origin. This supports the interpretation of TSPO PET as a microglia-specific neuroinflammatory biomarker in the primary tauopathy PSP.

We recognise the cellular substrate and mechanism driving increased TSPO radioligand binding might be disease-specific, with reports suggesting varying effects on TSPO expression across different diseases, species, and models[20,22,24–27]. Further work is required to exclude the possibility that the association between microglia and TSPO radioligand binding is PSP/tauopathy-specific, with PET-to-pathology comparisons needed in other neurodegenerative disorders. Few studies have reported TSPO expression in a range of microglial phenotypes[20,22,26] with a recent study showing an association with 'phagocytic' microglia[27]. Our finding that TSPO PET radioligand binding correlates with CD68+ microglia is aligned with the latter.

Our study has potential limitations, in relation to sample size, diagnostics and ligand specificity. Despite the relatively small sample size, we had adequate power, given (i) the large effect size expected from previous PET-only studies in PSP at the time of death (Cohen's *d* is often > 2)[8,10,28], and (ii) the use of a linear mixed-effect model for PET-to-pathology analyses, leveraging all nineteen regional data points available for the eight PSP donors. In addition, the correlation of TSPO PET to CD68+ microglia in every single case of our series, provides evidence to support the inference of generalisation[29]. Our study lacked power to confirm the mild effects of age- and sex on TSPO radioligand binding[6,30], but we note that the relationship between the PET and CD68 signals was present in all participants, regardless of age and sex.

PSP-Richardson's syndrome offers advantages as a demonstrator condition due to its strong clinicopathological correlation, relatively short PET-to-brain donation, and absence of a major vascular contributions, unlike AD. We used the first generation TSPO ligand [^{11}C]-PK11195.

While second (e.g., [^{11}C]-PBR28) and third (e.g., [^{18}F]-GE180) generation tracers may offer an improved signal-to-noise ratio[5], they are confounded by genetic polymorphisms affecting binding affinity. They are therefore less suitable for rare diseases, where gene-stratified recruitment would be especially challenging.

In summary, our findings support the use of TSPO PET as a microglia-specific neuroinflammatory biomarker in the primary tauopathy of PSP. Microglial TSPO levels contribute to *in vivo* [^{11}C]-PK11195 binding, over and above astrocytic levels. Given the PET evidence of increased TSPO radioligand binding in core pathological regions, and its predictive value for clinical decline, we suggest that TSPO PET can be used to quantify microglial-mediated neuroinflammation in primary tauopathies such as PSP, assisting the design of clinical trials with disease-modifying therapies.

Data availability

Anonymized *post-mortem* and PET data used for this analysis are available on request. Further participant-specific information, images or samples can be requested but are likely to require a data/material transfer agreement to adhere to consent restrictions including protection of confidentiality. For the purpose of open access, the authors have applied a Creative Commons Attribution (CC BY) license to any Author Accepted Manuscript version arising from this submission.

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Competing interests

The authors report no competing interests pertaining to this manuscript. MM has provided consultancy to Astex Pharmaceuticals; JBR has provided consultancy with Alector, Astex, Asceneuron, Astronautx, Curasen, CumulusNeuro, Eisai Ferrer, Invicro, Prevail, SVHealth; JOB has acted as a consultant for TauRx, Novo Nordisk, Biogen, Roche, Lilly and GE Healthcare and received grant and academic support from Avid/ Lilly, Merck and Alliance Medical. This is unrelated to the current work.

Supplementary material

Supplementary materials 1 and 2 are available at Brain online.

References

1. Wilson DM, Cookson MR, Van Den Bosch L, Zetterberg H, Holtzman DM, Dewachter I. Hallmarks of neurodegenerative diseases. *Cell*. 2023;186(4):693-714. doi:10.1016/j.cell.2022.12.032

2. Castro-Gomez S, Heneka MT. Innate immune activation in neurodegenerative diseases. *Immunity*. 2024;57(4):790-814. doi:10.1016/j.immuni.2024.03.010

3. Hartnell IJ, Blum D, Nicoll JAR, Dorothee G, Boche D. Glial cells and adaptive immunity in frontotemporal dementia with tau pathology. *Brain*. 2021;144(3):724-745. doi:10.1093/brain/awaa457

4. Odfalk KF, Bieniek KF, Hopp SC. Microglia: Friend and foe in tauopathy. *Prog Neurobiol*. 2022;216:102306. doi:10.1016/j.pneurobio.2022.102306

5. Malpetti M, Franzmeier N, Brendel M. PET Imaging to Measure Neuroinflammation In Vivo. In: Perneczky R, ed. *Biomarkers for Alzheimer's Disease Drug Development*. Springer US; 2024:177-193. doi:10.1007/978-1-0716-3774-6_12

6. De Picker LJ, Morrens M, Branchi I, et al. TSPO PET brain inflammation imaging: A transdiagnostic systematic review and meta-analysis of 156 case-control studies. *Brain Behav Immun*. 2023;113:415-431. doi:10.1016/j.bbi.2023.07.023

7. Palleis C, Sauerbeck J, Beyer L, et al. In Vivo Assessment of Neuroinflammation in 4-Repeat Tauopathies. *Movement Disorders*. 2021;36(4):883-894. doi:10.1002/mds.28395

8. Malpetti M, Passamonti L, Rittman T, et al. Neuroinflammation and Tau Colocalize in vivo in Progressive Supranuclear Palsy. *Annals of Neurology*. 2020;88(6):1194-1204. doi:10.1002/ana.25911

9. Malpetti M, Passamonti L, Jones PS, et al. Neuroinflammation predicts disease progression in progressive supranuclear palsy. *J Neurol Neurosurg Psychiatry*. 2021;92(7):769-775. doi:10.1136/jnnp-2020-325549

10. Passamonti L, Rodríguez PV, Hong YT, et al. [11C]PK11195 binding in Alzheimer disease and progressive supranuclear palsy. *Neurology*. 2018;90(22):e1989-e1996. doi:10.1212/WNL.0000000000005610

11. Malpetti M, Roemer SN, Harris S, et al. Neuroinflammation Parallels 18F-PI-2620 Positron Emission Tomography Patterns in Primary 4-Repeat Tauopathies. *Movement Disorders*. 2024;39(9):1480-1492. doi:10.1002/mds.29924

12. Malpetti M, Cope TE, Street D, et al. Microglial activation in the frontal cortex predicts cognitive decline in frontotemporal dementia. *Brain*. 2023;146(8):3221-3231. doi:10.1093/brain/awad078

13. Malpetti M, Kievit RA, Passamonti L, et al. Microglial activation and tau burden predict cognitive decline in Alzheimer's disease. *Brain*. 2020;143(5):1588-1602. doi:10.1093/brain/awaa088

14. Finze A, Biechele G, Rauchmann BS, et al. Individual regional associations between Aβ-, tau- and neurodegeneration (ATN) with microglial activation in patients with primary and secondary tauopathies. *Mol Psychiatry*. 2023;28(10):4438-4450. doi:10.1038/s41380-023-02188-8

15. Guilarte TR. TSPO in diverse CNS pathologies and psychiatric disease: A critical review and a way forward. *Pharmacology & Therapeutics*. 2019;194:44-58. doi:10.1016/j.pharmthera.2018.09.003

16. Banati RB, Myers R, Kreutzberg GW. PK ('peripheral benzodiazepine')--binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [3H]PK11195 binding to activated microglia. *J Neurocytol*. 1997;26(2):77-82. doi:10.1023/a:1018567510105

17. Guilarte TR, Kuhlmann AC, O'Callaghan JP, Miceli RC. Enhanced expression of peripheral benzodiazepine receptors in trimethyltin-exposed rat brain: a biomarker of neurotoxicity. *Neurotoxicology*. 1995;16(3):441-450.

18. Hannestad J, Gallezot JD, Schafbauer T, et al. Endotoxin-induced systemic inflammation activates microglia: [11C]PBR28 positron emission tomography in nonhuman primates. *NeuroImage*. 2012;63(1):232-239. doi:10.1016/j.neuroimage.2012.06.055

19. Yao R, Pan R, Shang C, et al. Translocator Protein 18 kDa (TSPO) Deficiency Inhibits Microglial Activation and Impairs Mitochondrial Function. *Front Pharmacol*. 2020;11. doi:10.3389/fphar.2020.00986

20. Nutma E, Fancy N, Weinert M, et al. Translocator protein is a marker of activated microglia in rodent models but not human neurodegenerative diseases. *Nat Commun*. 2023;14(1):5247. doi:10.1038/s41467-023-40937-z

21. Owen DR, Narayan N, Wells L, et al. Pro-inflammatory activation of primary microglia and macrophages increases 18 kDa translocator protein expression in rodents but not humans. *J Cereb Blood Flow Metab*. 2017;37(8):2679-2690. doi:10.1177/0271678X17710182

22. Nutma E, Stephenson JA, Gorter RP, et al. A quantitative neuropathological assessment of translocator protein expression in multiple sclerosis. *Brain*. 2019;142(11):3440-3455. doi:10.1093/brain/awz287

23. Kovacs GG, Lukic MJ, Irwin DJ, et al. Distribution patterns of tau pathology in progressive supranuclear palsy. *Acta Neuropathol*. 2020;140(2):99-119. doi:10.1007/s00401-020-02158-2

24. Gui Y, Marks JD, Das S, Hyman BT, Serrano-Pozo A. Characterization of the 18 kDa translocator protein (TSPO) expression in post-mortem normal and Alzheimer's disease brains. *Brain Pathology*. 2020;30(1):151-164. doi:10.1111/bpa.12763

25. Tournier BB, Tsartsalis S, Ceyzériat K, et al. Astrocytic TSPO Upregulation Appears Before Microglial TSPO in Alzheimer's Disease. *J Alzheimers Dis*. 2020;77(3):1043-1056. doi:10.3233/JAD-200136

26. Garland EF, Dennett O, Lau LC, et al. The mitochondrial protein TSPO in Alzheimer's disease: relation to the severity of AD pathology and the neuroinflammatory environment. *Journal of Neuroinflammation*. 2023;20(1):186. doi:10.1186/s12974-023-02869-9

27. Garland EF, Antony H, Kulagowska L, et al. The microglial translocator protein (TSPO) in Alzheimer's disease reflects a phagocytic phenotype. *Acta Neuropathol*. 2024;148(1):62. doi:10.1007/s00401-024-02822-x

28. Holland N, Jones PS, Savulich G, et al. Synaptic Loss in Primary Tauopathies Revealed by [C]UCB-J Positron Emission Tomography. *Movement Disorders*. 2020;35(10):1834-1842. doi:10.1002/mds.28188

29. Friston KJ, Holmes AP, Worsley KJ. How Many Subjects Constitute a Study? *NeuroImage*. 1999;10(1):1-5. doi:10.1006/nimg.1999.0439

30. Biechele G, Rauchmann BS, Janowitz D, et al. Associations between sex, body mass index and the individual microglial response in Alzheimer's disease. *Journal of Neuroinflammation*. 2024;21(1):30. doi:10.1186/s12974-024-03020-y

Figure legends

Figure 1: TSPO multicellular expression in human PSP *post-mortem* brain. (A) TSPO immunohistochemistry in frontal lobe tissue. **(B)** Immunofluorescence images of TSPO (red), microglial marker IBA1 (green), and DAPI (blue). **(C, D)** Insets of (B) showing individual channels for (C) IBA1 and (D) TSPO. **(E)** Immunofluorescence images of TSPO and endothelial marker CD31. **(F)** Immunofluorescence images of TSPO (red), astrocytic marker GFAP (green), and DAPI (blue). **(G, H)** Insets of (F) showing individual channels for (G) GFAP and (H) TSPO.

Figure 2: TSPO expression in people with PSP vs. controls. (A) Total TSPO area fraction. **(B, D)** Area fraction of TSPO co-localised with (B) IBA1 (microglia) or (D) GFAP (astrocytes) (Mann-Whitney Test; $p < 0.05$; ns = not significant; mean ± SD). **(C, E)** TSPO co-localisation with (C) IBA1 or (E) GFAP in grey (GM) and white matter (WM) (Kruskal-Wallis Test; PSP GM vs. WM, $p < 0.01$). **(F, G)** TSPO per microglia quantification (Mann-Whitney Test; $p < 0.05$). **(H)** Microglial TSPO vs. total microglia in PSP (red) and controls (blue), grey (circles) and white (triangles) matter (Spearman's Correlation; $p < 0.05$).

Figure 3: PET to *post-mortem* correlation of [11C]-PK11195 BP_{ND} with CD68+ microglia and TSPO cell-type specific expression. (A) CD68+ microglia area fractions by region in WM (blue), GM (green), and subcortical structures (red). **(B)** Regional [11C]-PK11195 binding potential (BP_{ND}) in the corresponding compartments. **(C)** Association between regional CD68 area fractions and *ante-mortem* [11C]-PK11195 BP_{ND}. Dots represent individual regional values; black line shows the group-level association; coloured lines represent patient-specific associations, estimated by the model. **(D, E)** [11C]-PK11195 BP_{ND} association with (D) TSPO-IBA1 and (E) TSPO-GFAP co-localisation area fractions in posterior frontal cortex (BA6) across GM (circles) and WM (triangles).