

TSPO postmortem expression patterns in cognitively unimpaired and mild cognitive impairment participants

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ABSTRACT

Background: ER176 is a positron emission tomography (PET) tracer used to image neuroinflammation by binding to the translocator protein (TSPO). While TSPO is elevated in activated microglia, its expression across different cognitive states in the human brain remains poorly characterized. Our goal was to characterize the cellular expression of TSPO in the postmortem brain.

Methods: We performed multiplex immunofluorescence on amygdala and hippocampal sections from three postmortem cases with antemortem [¹¹C] ER176 PET imaging. Subjects were genotyped for the *TSPO* rs6971 polymorphism and classified as high-affinity binders (G/G), mixed-affinity binders (A/G), or low-affinity binders (A/A). Cases are reported as cognitive status at time of imaging, neuropath diagnosis, and *TSPO* genotype: (1) cognitively unimpaired (CU), primary age related tauopathy (PART), A/G; (2) mild cognitive impairment (MCI), limbic predominant age-related TDP-43 encephalopathy (LATE), A/G; and (3) MCI, Alzheimer's disease (AD), G/G. Sections were stained for TSPO, microglial marker IBA1, and nuclear marker DAPI.

Results: TSPO expression was detected in microglia, endothelial cells (including blood vessels and choroid plexus), astrocytes, and neurons (**Figure 1**). In microglia, TSPO did not fully overlap with IBA1-positive areas. In the CU/PART subject, neuronal TSPO staining was faint or absent (**Figure 2**), whereas the MCI/LATE and AD subjects showed more intense and frequent neuronal TSPO staining. Interestingly, the AD case with a high-affinity binding genotype (G/G) had fewer microglia overall, as identified by IBA1 staining, compared to mixed-affinity binding cases (A/G). Given that ER176 is used to image neuroinflammation via TSPO, this observation may reflect a paradox between binding affinity and microglial abundance, warranting further investigation.

Conclusions: This preliminary study demonstrates that TSPO is expressed across multiple cell types with variability in expression levels. These findings underscore the importance of further characterizing TSPO distribution in healthy and neurodegenerative conditions. Future work will expand multiplex immunofluorescence to additional cell markers and cases and explore correlations between TSPO staining and ER176 PET signal.

CELLULAR EXPRESSION OF TSPO

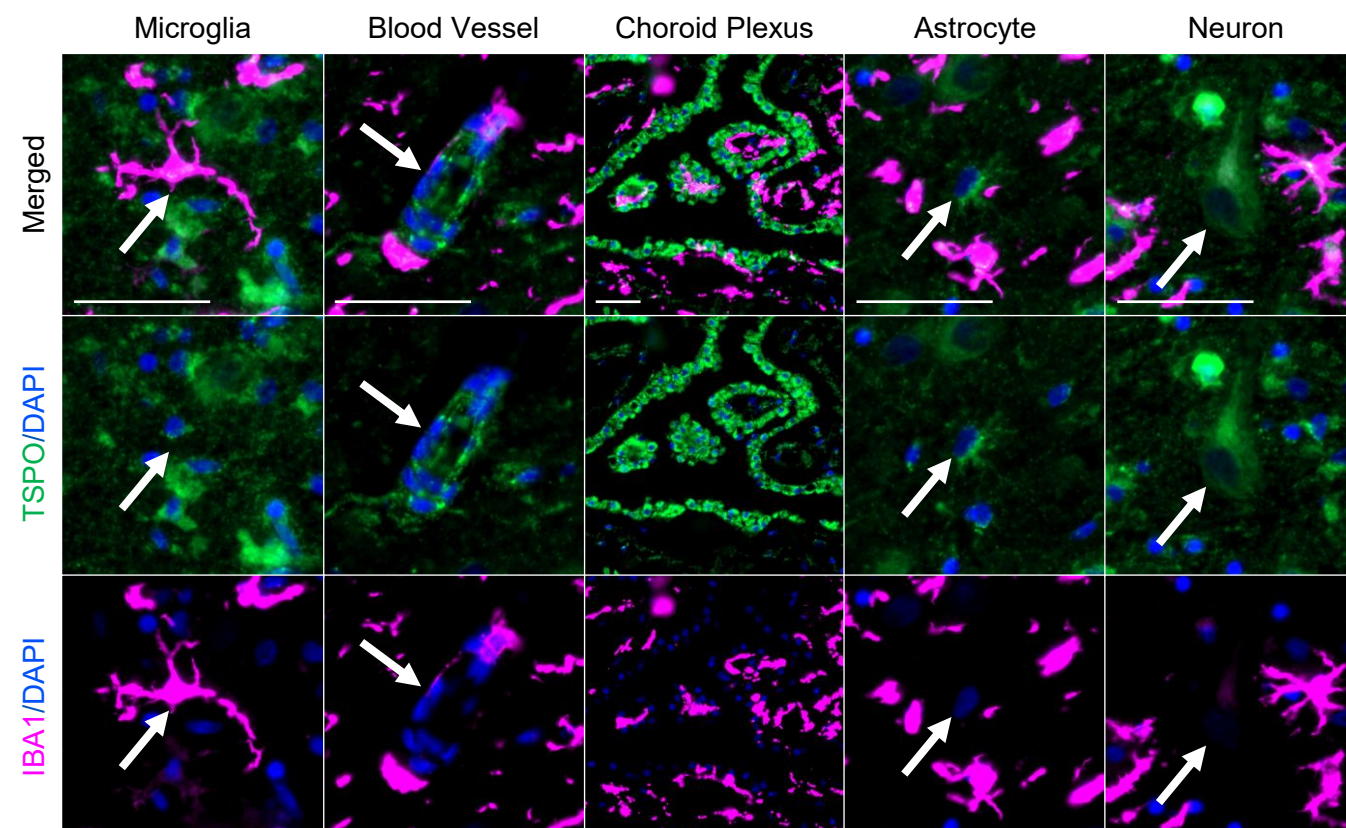


Figure 1. TSPO expression in multiple cell types. TSPO positivity (green) was observed in microglia (IBA1 positive, pink), blood vessel endothelia, choroid plexus endothelia, astrocytes, and neurons. Microglia, blood vessel, astrocyte, and neuron images were obtained from the mild cognitive impaired/limbic predominant age-related TDP-43 encephalopathy case (MCI/LATE), and the choroid plexus image was obtained from the cognitively unimpaired/primary age related tauopathy case (CU/PART). Scale bar measures 50 μ m.

TSPO AND IBA1 EXPRESSION IN CU AND MCI CASES

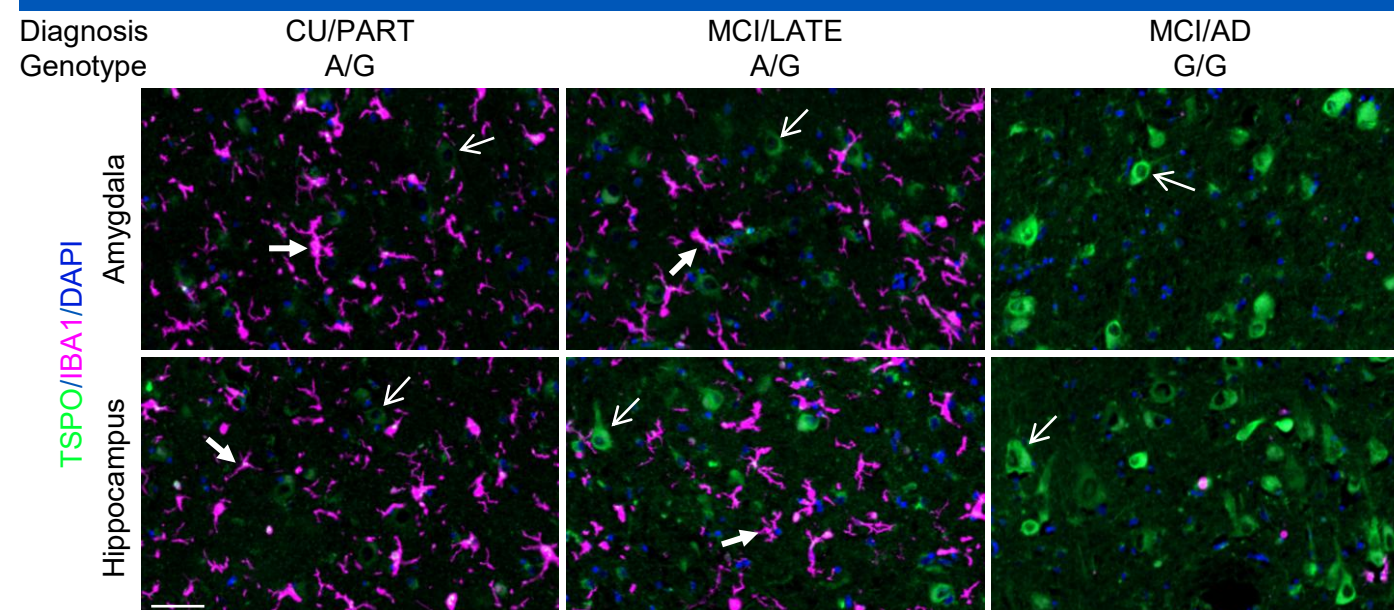


Figure 2. TSPO expression in amygdala and subiculum of the hippocampus. Images from multiplex immunofluorescence showing translocator protein (TSPO, green), microglia (IBA1, pink, closed arrowhead), and nuclei (DAPI, blue). TSPO more strongly stained neurons (open arrowhead) in mild cognitive impairment (MCI) cases compared to the cognitively unimpaired/primary age related tauopathy case (CU/PART). IBA1 expression was reduced in the high-affinity binding genotype compared to mixed-affinity binding genotype. Scale bar measures 50 μ m.

CONCLUSIONS

- TSPO was identified in multiple cell types, including microglia, endothelial cells, astrocytes, and neurons
- TSPO appeared increased in neurons MCI/LATE and AD cases compared to the CU/PART case
- Future studies include expanding multiplex immunofluorescence and exploring correlations to ER176 PET
- Potential paradox between microglial abundance binding affinity genotypes warrants further investigation

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