Variation on endothelial tight junctions in the TgF344-AD rat in Alzheimer’s disease
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Background and Rationale
Over half of adults over the age of 60 develop dementia, including Alzheimer’s disease (AD), with the primary risk factor being age—whose cause is still largely unknown. Age-associated vascular inflammation is hypothesized to contribute to AD development, as the neurovascular inflammatory response creates a ripe environment for neurodegenerative disease. Disruption of the blood brain barrier (BBB) leaves potential for increased neuron and glial injury as a result of increased efflux of toxic chemicals from the vasculature, as well as reduced metabolites accessible to these now-vulnerable cells. Given that the effectiveness of the BBB decreases with age, it is worth investigating what aspects of BBB function change with time in an AD-like phenotype.

Endothelial tight junction (TJ) proteins strictly regulate influx and efflux of plasma proteins such as albumin, immunoglobulin, and fibrinogen; dysfunction of these TJs would suggest paracellular openings that allow serum proteins to leak into the brain parenchyma, potentially causing cytotoxicity.

Methods
This study leveraged the rat model TgF344-AD which recapitulates the inflammatory responses seen in AD-like clinical features due to the overexpression of the human mutant amyloid precursor protein and presenilin 1 genes. Age- and sex-matched wildtype (WT) and TgF344-AD (Tg) rats were raised for brain tissue collection at 10 months of age (n=10 total; n=4/4 in the CA1 and CA3 regions) and at 15 months of age (n=9 total; n=4/4 in the CA1 and CA3 regions). Tissues were cryosectioned at 10 mm at ~bregma -4.5 mm to reveal the hippocampus in its entirety, the prefrontal cortex, and perform and entorhinal cortices for later data collection.

Immunohistochemistry (IHC) was performed on at least one tissue from each animal. Stains included occludin (OCCLN) and Claudin-5 (CLDN5) and amyloid-β (OC) deposition as a control to confirm an AD phenotype. Claudin-5 and occludin were stained on the same tissue for relevant quantitative analysis, and amyloid-β staining occurred on a separate section of similar bregma. Amyloid-β antibodies were monoclonal, Claudin-5 antibodies were monoclonal, and occludin antibodies were polyclonal; all tissues were treated with DAPI for nuclear staining.

Using the ImageXpress® Micro by Molecular Devices, the hippocampal regions of each tissue were imaged at 20x. IHC images were cropped by a blinded researcher to isolate hippocampal regions of interest (CA1, CA3, and the dentate gyrus). Cropped images were analyzed by generating a binary image using a custom analysis module.

Data were exported, compiled, and analyzed. Amyloid-β was analyzed for area via a two-tailed t test and tight junction proteins were analyzed for average ratio of immunofluorescence via multiple unpaired tests with Welch correction and multiple comparisons for false discovery rate.

Conclusions and Future Directions
Amyloid-β deposition: TgF344-AD brains at 15 months have significantly more amyloid-β plaques, confirming an Alzheimer-like phenotype, and display regional heterogeneity throughout the brain. Claudin-5 tight junctions were significantly decreased in transgenic brains at both 10 and 15 months of age; strongly vascularized regions of the hippocampus displayed highly decreased claudin-5 levels at both age points. Tg433-AD rats at age points 10 and 15 months of age had significantly more claudin-5 in the CA1, CA3, and dentate gyrus regions of the hippocampus.

Future directions: Optimization of background removal in occludin immunohistochemistry, as the commonly-used polyclonal antibody has been observed in non-endothelial tight junctions in brain matter. Further data retrieval to assess regional heterogeneity of (1) tight junction expression (2) gels and (3) aquaporin-4 translocation in hippocampal, prefrontal, and piriform tissues across phenotypes and time.