Variation on endothelial tight junctions in the TgF344-AD rat in Alzheimer's disease

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UCDAVIS VETERINARY MEDICINE
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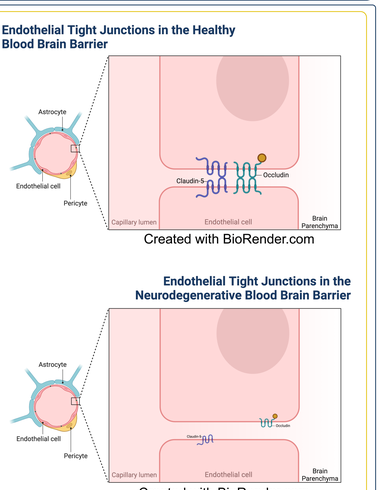
Background and Rationale

Over half of adults over the age of 80 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 neuro-degenerative disease.

Disruption of the blood brain barrier(BBB) leaves potential for increased neuronal 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.



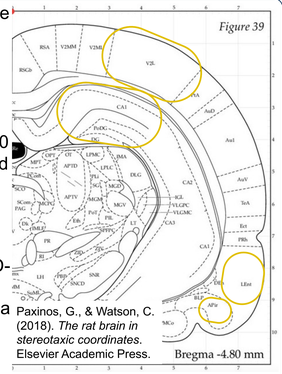
Note: Changes in of tight junctions represent a decrease in quantity, not protein size.

Hypothesis

Tight junction proteins claudin-5 and occludin will be decreased in brains that display AD-like phenotypes. AD-like phenotypes present with regional heterogeneity, brain regions are not equally affected by AD; specifically, claudin-5 and occludin will be decreased in highly vascularized regions of the hippocampus (CA1, CA3, and the dentate gyrus). Claudin-5 and occludin will also decrease in these regions over time.

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 presinilin 1 genes. Age- and sex-matched wildtype (Wt) and TgF344-AD (Tg) rats were reared for brain tissue collection at 10 months of age (n=10 total; n_{Wt}=4 & n_{Tg}=6) and at 15 months of age (n=9 total; n_{Wt}=4 & n_{Tg}=5). Tissues were cry sectioned at 10mm at ~bregma -4.80 mm to reveal the hippocampus in its entirety, the prefrontal cortex, and piriform and entorhinal cortices for later data collection.



Paxinos, G., & Watson, C. (2018). *The rat brain in stereotaxic coordinates.* Elsevier Academic Press.

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 mask using an intensity threshold dictated by a negative (no primary antibody) and positive control sample. Binary masks were used to calculate immunopositive area and average intensities are calculated by 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 t tests with Welch correction and multiple comparisons for false discovery rate.

Results

Amyloid-β Deposition

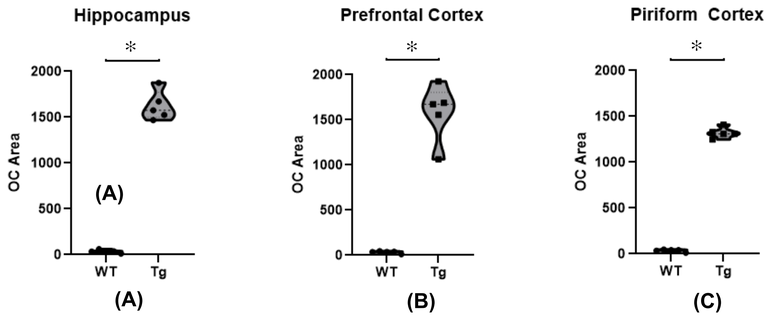


Figure 1 | **[type of plot]** quantifying the area of amyloid-β (OC) immunofluorescent area present in **(A)** the hippocampus, **(B)** the prefrontal cortex, and **(C)** the piriform cortex in wildtype and transgenic (Tg) Tg433-AD rats aged to 15 months. ✻ = p≤0.05; Hippocampal, prefrontal cortical, and piriform cortical tissues had significantly more amyloid-β plaques in transgenic brains than in wildtype brains.

Claudin-5 Quantitative Immunoreactivity

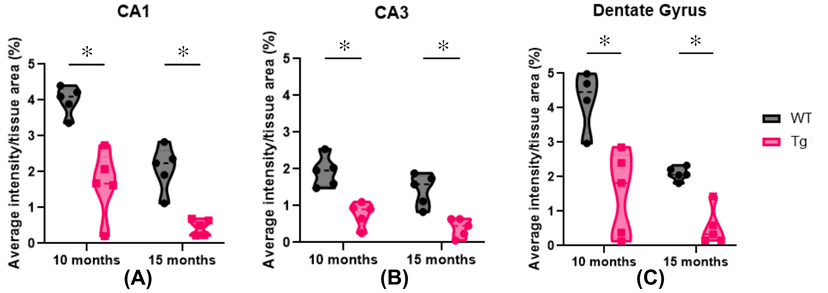


Figure 2 | **[type of plot]** quantifying the average ratio of immunofluorescent claudin-5 proteins present in **(A)** the CA1 region of the hippocampus, **(B)** the CA3 region of the hippocampus, and **(C)** the dentate gyrus region of the hippocampus in wildtype and transgenic (Tg) Tg433-AD rats at age points 10 and 15 months. ✻ = p≤0.05; Transgenic 10- and 15-month-old brains had significantly more claudin-5 in the CA1, CA3, and dentate gyrus regions of the hippocampus.

Occludin Quantitative Immunoreactivity

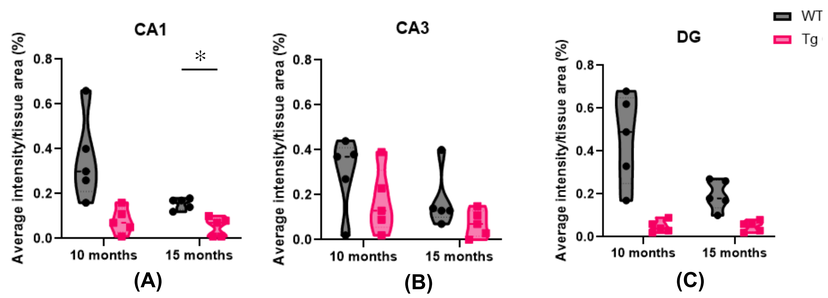
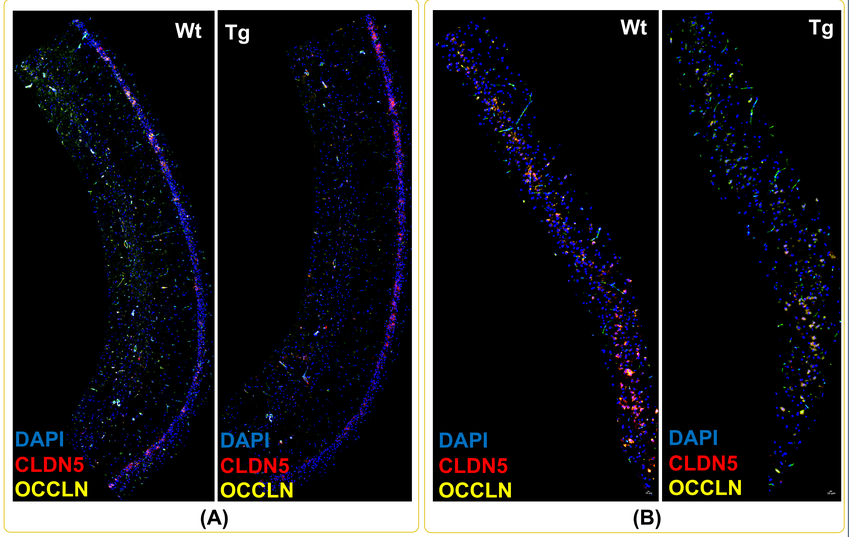


Figure 3 | **[type of plot]** quantifying the average ratio of immunofluorescent occludin proteins present in **(A)** the CA1 region of the hippocampus, **(B)** the CA3 region of the hippocampus, and **(C)** the dentate gyrus region of the hippocampus in wildtype and transgenic (Tg) Tg433-AD rats at age points 10 and 15 months. ✻ = p≤0.05; Transgenic 15-month-old brains had significantly more occludin in the CA1 region of the hippocampus.

Tight Junction Qualitative Immunoreactivity



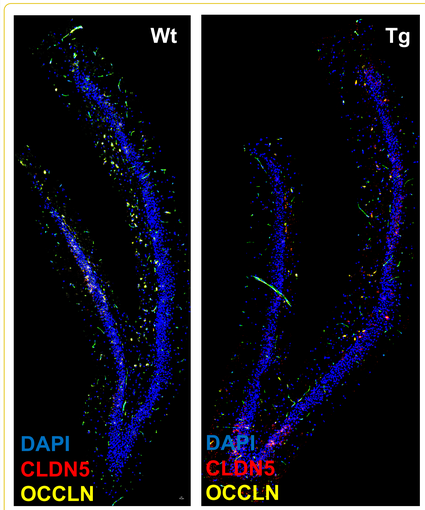


Figure 4 | Quantitative data from immunohistochemistry staining for nuclei (blue), claudin-5 (red), and occludin (yellow) in **(A)** the CA1 region of the hippocampus, **(B)** the CA3 region of the hippocampus, and **(C)** the dentate gyrus region of the hippocampus in wildtype (Wt) and transgenic (Tg) Tg433-AD rats at 15 months of age. Overlapped regions of claudin-5 and occludin appear green. Overlapped regions of nuclei, claudin-5, and occludin appear cyan.

Conclusions and Future Directions

Amyloid-β deposition: TgF344AD 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 the highly vascular CA1, CA3, and dentate gyrus hippocampal regions.
Occludin tight junctions were significantly decreased in the highly vascular CA1 hippocampal region at 15 months of age.

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) gliosis and (3) aquaporin-4 translocation in hippocampal, prefrontal, and piriform tissues across phenotypes and time.

Acknowledgements

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