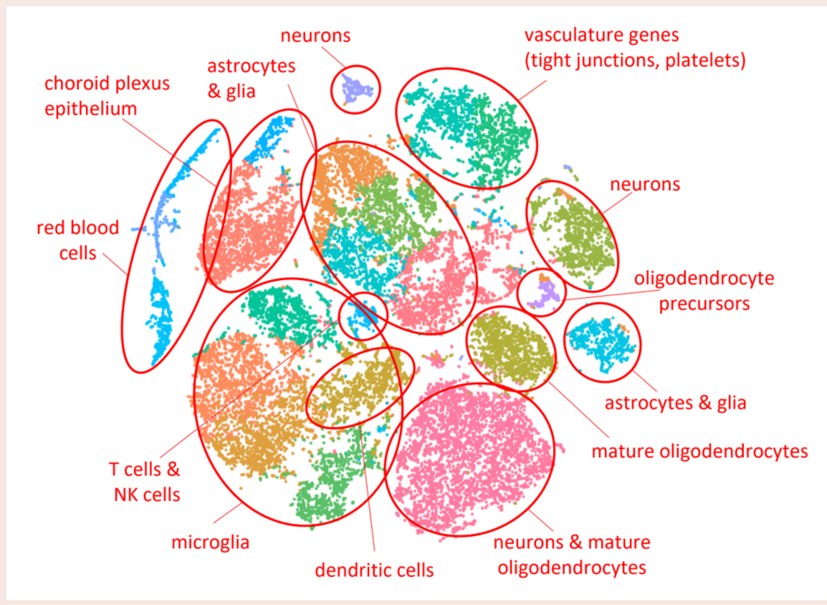
Novel **single-cell RNA-sequencing** data suggests upregulation of **angiogenesis**-associated **gene pathways** in a late-stage mouse model of **Alzheimer's Disease**.



Developing a transcriptomic atlas of Alzheimer's Disease progression in the Tg2576 mouse model using single-cell RNA-sequencing technology

Authors & Affiliations:
Kelly Marie Johns[1-5], Giorgia Caspani[1,3-5], Anne Haegert[2], Maria-Elizabeth Baeva[1,3,4,6], Chaahat Singh[1-5], Angela Mathews[6], Stephane Le Bihan[2], Cheryl Pfeifer[1-8], Colin Collins[2,8], Wilf Jefferies[1-8].
1) Michael Smith Laboratories, 2) The Vancouver Prostate Centre 3) Centre for Blood Research, 4) The Djavad Mowafaghian Centre for Brain Health, 5) Department of Medical Genetics, 6) Department of Microbiology and Immunology, 7) Department of Zoology, 8) Department of Urologic Sciences.

BACKGROUND

- Alzheimer's disease (AD) is a debilitating neurodegenerative condition characterized by cognitive decline, progressing to eventual dementia and death.
- Recent discoveries have challenged the traditional "amyloid hypothesis" of AD, warranting new approaches and prospective treatment agents in the AD therapeutic pipeline.
- Angiogenesis, the formation of new blood vessels, is characteristic of early AD. Early evidence suggests that modulating angiogenesis may repair damage in the AD brain, though transcriptomic data is currently lacking.
- AIM: to create the first single-cell view of AD progression in mice in order to identify alternative mechanisms of AD pathophysiology, and reveal putative molecular targets for AD drug therapy.

METHODS

- Tg2576 mice contain a mutation in the Amyloid Precursor Protein (*APP)* gene which results in a double amino-acid substitution. In Tg2576 mice, expression of mutated APP reliably causes overexpression of human APP in neurons, resulting in AD pathophysiology.
- Single-cell RNA-sequencing (scRNA-seq) enables gene expression profiling from individual cells in a tissue to define the specific contribution of each cell to the function of the whole tissue.

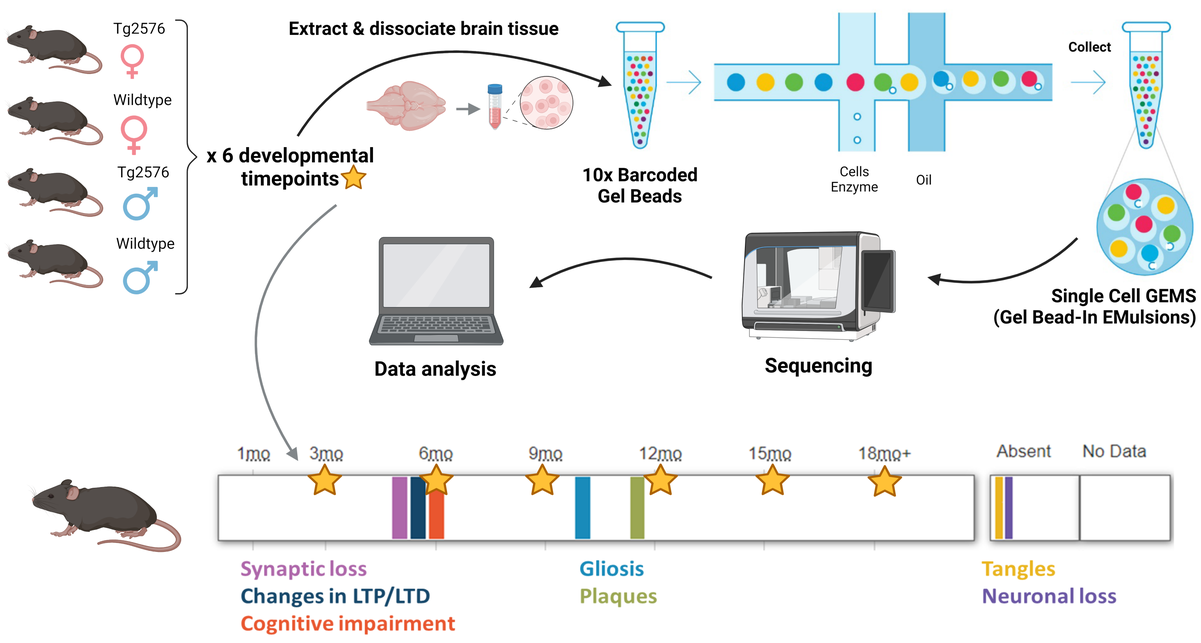


Figure 1: Experimental methods diagram. Cohorts of four mice representing both AD and control genotypes (Tg2576 and wildtype) and both sexes (male and female) were humanely euthanized at six different developmental timepoints (N=24). Brains were dissected and dissociated into a single-cell suspension. Individual cells were captured, and cellular mRNA was sequenced and profiled at a single cell resolution. Single-cell expression data was analyzed using 10X Genomics software: data was pre-processed with Cell Ranger then imported into Loupe Browser for differential expression analysis.

PRELIMINARY RESULTS

- Preliminary data on the 18+ month cohort demonstrates clear clusters of different cell populations (N=4).
- Pathway analysis demonstrated statistically significant enrichment in pathways related to angiogenesis ($p < 0.001$), VEGF production ($p = 0.011$) and endothelial cell proliferation ($p = 0.042$).

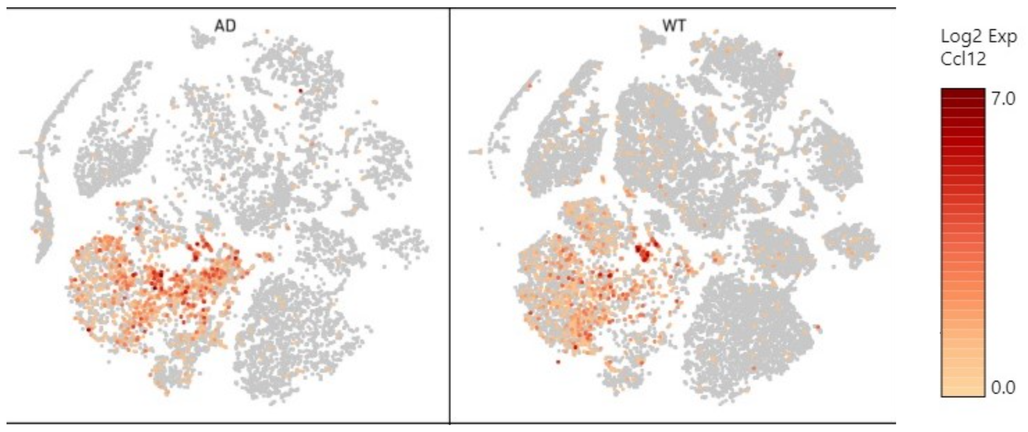


Figure 2: sc-RNA-seq gene expression analysis illustrates an upregulation in the *CCL12* gene, a key promoter of angiogenesis, in Tg2576 mice (P = 6.09E-7). C-C Motif Chemokine Ligand 12 (CCL12*)* is a signaling protein that induces directional movement of leukocytes, and various endothelial and epithelial cells. Gene Ontology terms associated with *CCL12* include: angiogenesis, inflammatory response, and monocyte chemotaxis.

CONCLUSION

- This study offers a unique cellular-level view of transcriptional alterations associated with AD pathology in mice. Our methods will capture:
  - Cell-type-specific and shared gene-expression perturbations
  - Disease-associated cellular subpopulations
  - Sex-biased transcriptional responses.
- Preliminary findings support the 'vascular angiogenesis model' for AD
  - Supports growing evidence that neo-angiogensis-induced disruptions to BBB integrity lead to the increase in amyloid-beta accumulation and subsequent AD pathology seen in Tg2576 mice and AD patients.
- Next steps:
  - Long-range and deep sequencing on Tg2576 samples to identify precise splice variants differentially expressed in more AD cells.
  - Cross-species validation experiments using various neural organoid "mini-brain" genetic models of AD.
  - Knock-out studies in rodent and neural organoid models of AD to probe differentially-expressed genes of interest.
  - Pre-clinical drug repurposing of FDA-approved medications with angiogenesis-modifying properties for treatment and prevention of AD using scRNA-seq as a readout/endpoint.

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