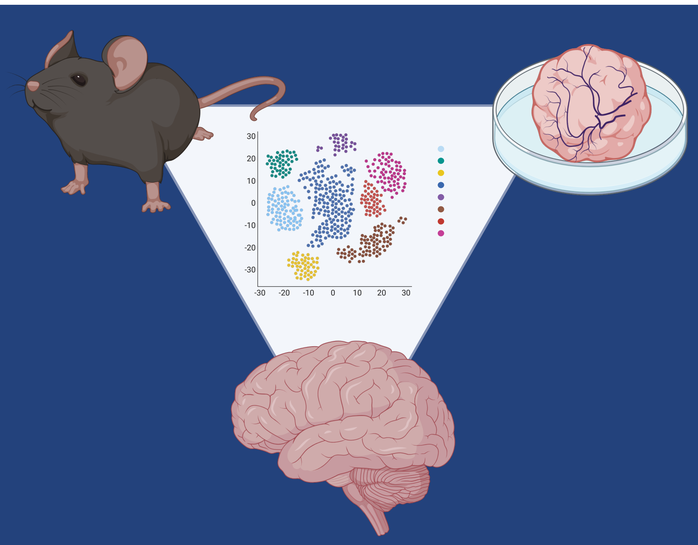
Novel **single-cell RNA-sequencing** data suggests upregulation of **angiogenesis**-associated **gene pathways** in **Alzheimer's Disease**.



Leveraging tri-tissue transcriptomics to elucidate neurovascular pathophysiology in Alzheimer's Disease

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BACKGROUND

- Alzheimer's disease (AD) is the most common form of dementia, affecting millions of people worldwide.
- Recent discoveries have challenged the traditional "amyloid hypothesis" of AD, warranting new approaches and prospective treatment agents in the AD therapeutic pipeline.
- An emerging "neurovascular theory" suggests that dysfunctional blood vessels may contribute to amyloid plaque formation , and indicates that modulating angiogenesis could be a potential therapeutic approach for treating AD.
- In order to probe the neurovascular theory in AD pathogenesis, I aim to perform scRNA-seq in three different tissue types:
  - Tg2576 (AD model) mouse brains
  - hPSC-derived neurovascular organoids
  - post-mortem tissue from AD patients
- This comprehensive approach is poised to unravel the precise molecular mechanisms underpinning AD's pathogenesis. In doing so, it holds the promise to unveil transformative treatments, potentially culminating in curative therapies for this devastating and fatal disease.

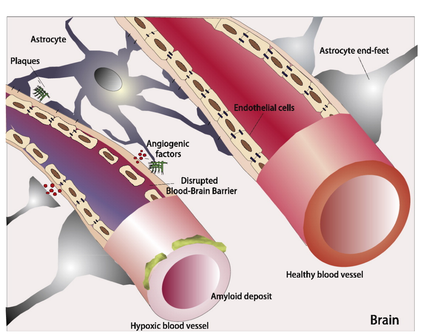

Figure 1: The neurovascular theory of AD
A key factor contributing to the development of AD is the malfunctioning of cerebral blood vessels, leading to reduced blood flow in the brain. Additionally, the weakening of the blood-brain barrier's (BBB) protective role results in the inadequate removal of harmful amyloid beta (Aβ) proteins, leading to vascular disturbances and a decrease in cognitive abilities.
Image source: Chakraborty et al. (2017)

METHODS

- 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.
- 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.

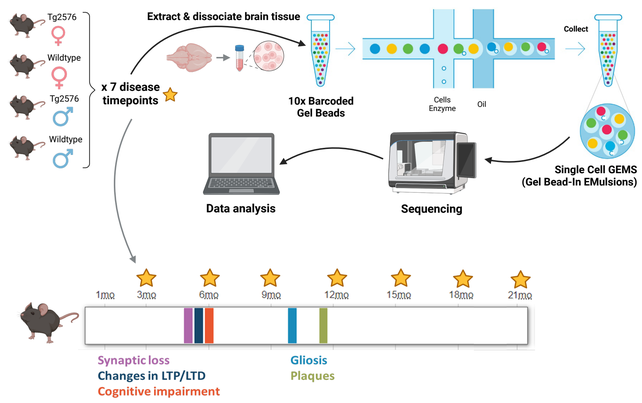

Figure 2: Experimental methods diagram for mouse studies.
Cohorts of four mice representing both AD and control genotypes (Tg2576 and wildtype) and both sexes (male and female) were humanely euthanized at six different disease progression timepoints (N=24). Brains were dissected and dissociated into 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.

- Neural organoids aka "mini brains are self-assembled 3D cellular aggregate generated from human pluripotent stem cells (hPSCs). They make for high-fidelity *in-vitro* models of human disease.
- Isogenic cell lines sourced from a human Klinefelter syndrome patient will be utilized to represent both female (XX) and male (XY) biological sexes on the same genetic background, allowing for the precise identification of sex differences in AD.

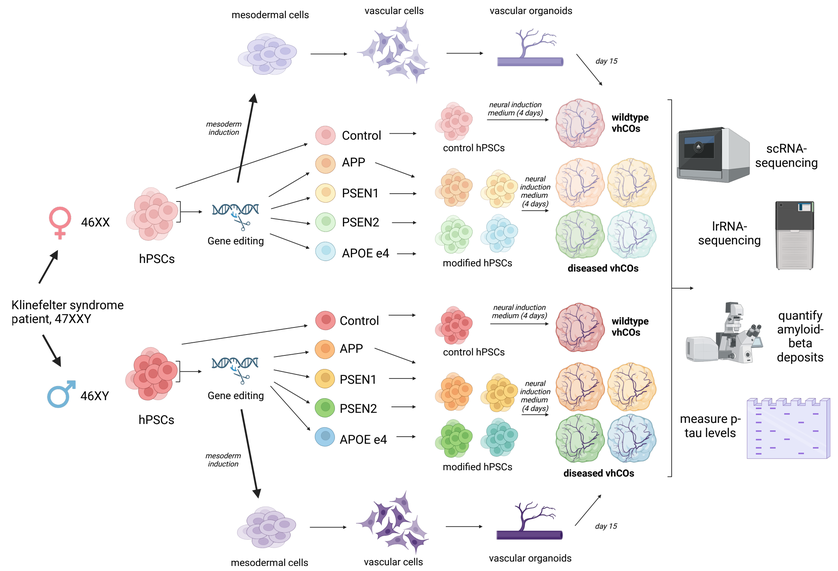

Figure 3: AD neural organoid workflow.
Prior to co-culturing, four known AD mutations will be introduced to cell lines fated for hCO development using CRISPR-Cas9 technology. BVO-fated hPSCs will be induced to form mesoderm, subsequently differentiated into vascular cells, separated into individual organoids, then maintained in 96-well plates until day 15. CO-fated hPSCs will be incubated for four days in neural induction medium then dissociated and co-cultured with 15-day-old BVO cells for seven days. On day 13 of CO growth, the organoid co-culture will be transferred to an orbital shaker in neural maturation medium for aging.

CONCLUSION

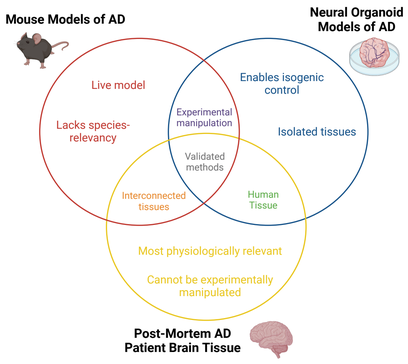

Figure 6: Venn Diagram depicting the benefits and limitations of each tissue type
Integrating three datasets from different tissues will balance the limitations of each tissue in order to tease out the most likely pathophysiological causes of AD

- This study offers a unique cellular-level view of transcriptional alterations associated with AD pathology in mouse and human tissue. 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 mouse brain samples to identify precise splice variants differentially expressed in AD cells.
  - Cross-species validation experiments using various neural organoid "mini-brain" genetic models of AD.
  - 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.

RESULTS

Pathway analysis demonstrated statistically significant enrichment in pathways related to: increased angiogenesis (p = 3.37E-02), vasculature development (p = 3.71E-02), and amyloid-beta formation (p = 1.49E-02).

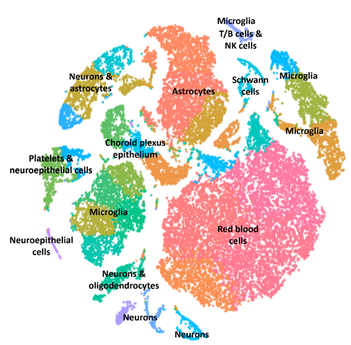

Figure 4: Cluster diagram for merged AD and wt samples.
During the data analysis process, clusters are formed by grouping cells with similar transcriptomic profiles. (N=8)

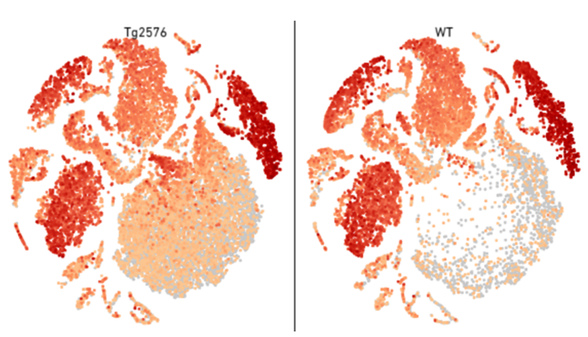

Figure 5: Representative gene comparison.
This representative gene, a negative regulator of angiogenesis, is upregulated in AD vs. wildtype mouse brains (*N=8*) *(P=2.82E-08).*

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