# Transcriptomic Atlas of Aged Human Brain Immune Cells: Unraveling Pathology-Associated Changes in Alzheimer's Disease

*Sudhagar Babu[1], Shihan Wang[1,2], Hongyi Zhao[1,2], Alexander K. Kusisky[1], Kimia Ghafari[1], Hansruedi Mathys[1]*
[1]Department of Neurobiology, University of Pittsburgh, PA, USA
[2]Tsinghua University, Beijing, China

University of Pittsburgh

Mathys Lab
Website

Center for Neuroscience
UNIVERSITY OF PITTSBURGH

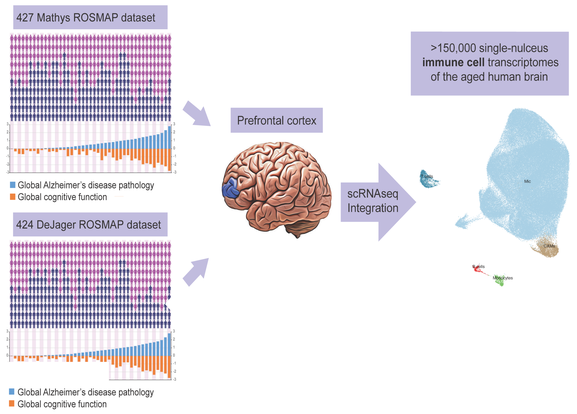
## INTRODUCTION & OBJECTIVES

**Alzheimer's disease (AD)**, a formidable challenge in the realms of aging and neurodegeneration, continues to demand comprehensive investigation to decipher its pathogenic mechanisms. While the aggregation of amyloid-beta plaques and tau tangles has been extensively studied, the intricate involvement of non-neuronal cells, especially **microglia** and their roles in **neuroinflammation**, remains a focal point of interest. Progress in **single-nucleus RNA sequencing (snRNAseq)** technology and analysis software has opened up new possibilities, enabling a comprehensive study of cellular diversity across millions of brain cells simultaneously. In this project, we embark on an exploration of microglia, with a particular emphasis on their subtypes within the **prefrontal cortex (PFC),** a region essential for cognitive function and significantly affected in AD. Our primary objective is to **unveil the molecular diversity and functional roles of distinct microglial subtypes throughout the course of AD**, offering insights into potential therapeutic targets and strategies for combating this devastating condition. By scrutinizing microglia at the single-cell level in a large sample from the **ROSMAP cohort[1]**, we aspire to **provide a deeper understanding of the complex cellular dynamics that underlie the disease, ultimately paving the way for more effective treatment modalities and novel targets**.

## MATERIALS & METHODS

In this study, we analyzed **over 150,000 immune cells** from **post-mortem DLPFC samples from more than 600 individuals** across **two ROSMAP datasets[2,3]** with individuals exhibiting **varying levels of AD pathologies.**

**Single-nucleus atlas of immune cells from the aged human prefrontal cortex across 615 individuals**




*Methods Graphical Summary:* **ROSMAP snRNAseq data acquisition.** Based on our recently published work[2], prefrontal cortical tissue from 427 participants in the ROSMAP cohort was combined with another recently published ROSMAP cohort consisting of 424 individuals[3]. The integrated dataset contains **615 individuals** with over 150,000 immune cells.

***Computational Analysis Pipeline Graphical Summary:***
Single Cell datasets objects were integrated using Seurat v5 (RPCA method). The integrated dataset were integrated using defaults setting and clustered using Leiden clustering algorithm.

We identified **14 transcriptomically distinct microglial cell states** including: homeostatic, inflammatory, phagocytic, glycolytic, stressed cell states, etc.

## RESULTS

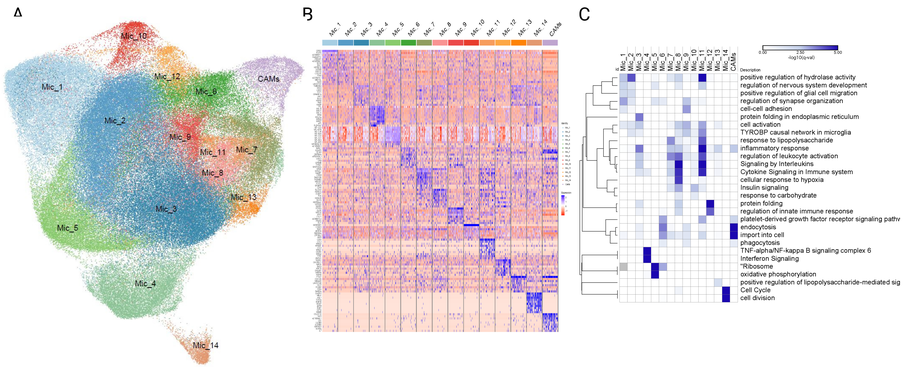



**Figure 1: Microglial cell states in aged human brains PFC.**
A). UMAP showing microglial cell states (154482 nuclei) Identified in PFC615 integrated ROSMAP dataset. B). Gene expression of marker genes across cell states. C). Gene ontology/pathway enrichment analysis of top 100 markers genes of microglial cell states.

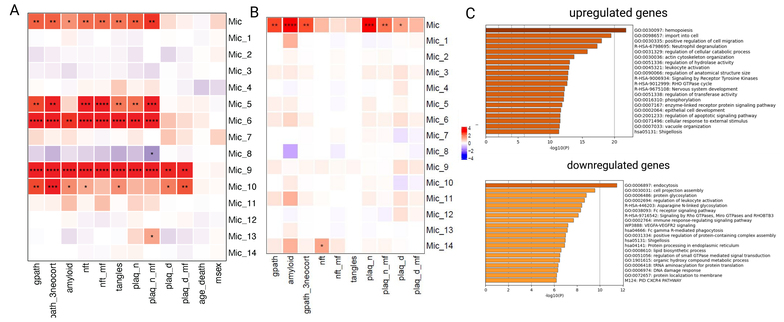



**Figure 2: Compositional changes and differential expression analysis of microglia in AD**
A). Compositional changes of microglial cell states in AD . B) Compositional changes of microglial senescent cells in AD. C). Gene ontology enrichment analysis of genes associated with global AD pathology

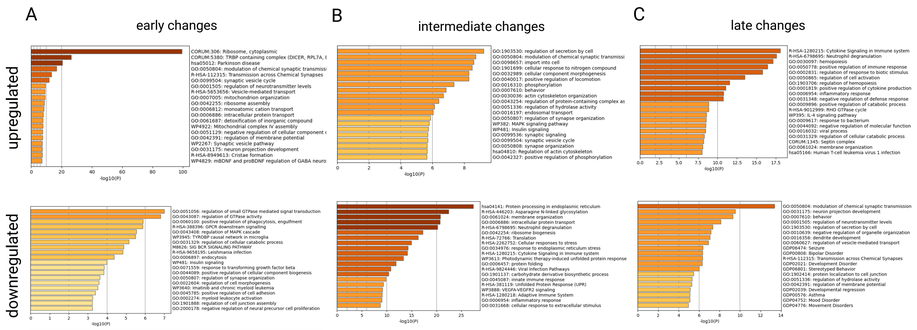



**Figure 3:** Temporal changes in gene expression across multiple stages of Alzheimer's disease. Gene ontology enrichment analysis of genes associated with global AD pathology in A). early B).intermediate and C). late stages of AD.

## RESULTS

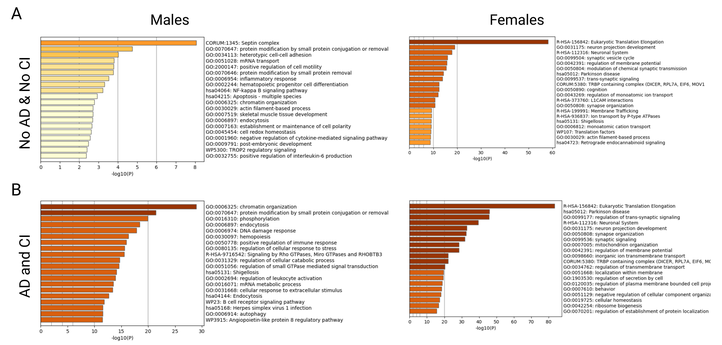



**Figure 4: Sex differences in microglia.**
A). Gene ontology enrichment analysis of **upregulated** differentially expressed genes between **male** and **female individuals** without AD or cognitive impairment (No AD & No CI).
B) Gene ontology enrichment analysis of **upregulated** differentially expressed genes between **male** and **female individuals** with AD and cognitive impairment (AD & CI).

## CONCLUSIONS

A number of new transcriptomic changes occurring in **microglia** in the **prefrontal cortex** of **AD patients** were identified:

- **Microglia cell compositions** are **increased** in AD
- **Phagocytic, lipid processing** and **stressed** microglia are associated with various **AD pathologies**
- There is a significant **enrichment** of **senescent microglial cells** in AD
- Genes related to **DNA damage** and **chromatin organization** processes are enriched in **males** whereas **mitochondrial** and **ribosomal dysfunction** genes are more pronounced in **females**

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