Exploring Astrocyte States and Altered Pathways in Alzheimer's Disease: A Transcriptomics Approach

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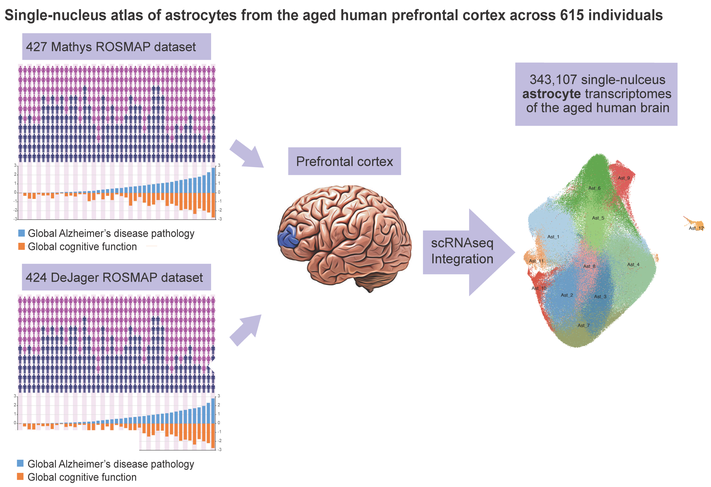
INTRODUCTION & OBJECTIVES

Alzheimer's disease (AD), a devastating neurodegenerative disorder, remains a substantial challenge in the field of neuroscience. Although the hallmark pathological features of the disease, such as amyloid-beta plaques and tau tangles, have been extensively studied, the role of non-neuronal cells, particularly **astrocytes**, in the progression of the disease is still not fully understood. Advances in **single-nucleus RNA sequencing (snRNAseq)** technology and software have enabled the exploration of cellular heterogeneity across millions of cells within the brain. In this paper, we delve into the intricate world of astrocytes, focusing on their subtypes within the **prefrontal cortex (PFC)**, a region essential for higher cognitive functions and significantly affected in AD. Analyzing a large sample of individuals from the **ROSMAP cohort**[1], **we unravel the correlations between molecularly and functionally diverse astrocyte subtypes throughout AD progression**, shedding light on alternative, non-neuronal strategies for studying and targeting this pervasive condition. By investigating the role of astrocytes at the single-cell level, we hope to provide a deeper understanding of the complex cellular dynamics that underlie AD and can be used to ultimately be used to develop more effective treatments.

MATERIALS & METHODS

In our study, we extensively analyzed **over 340,000 astrocytes** from **more than 600 post-mortem PFC brain samples** donated by individuals in the **ROSMAP cohort**. Individuals had varying levels of **AD-related pathologies**.



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 **12 astrocyte cell states (clusters)** including: reactive, non-reactive, inflammatory, stressed cell states, etc.

RESULTS

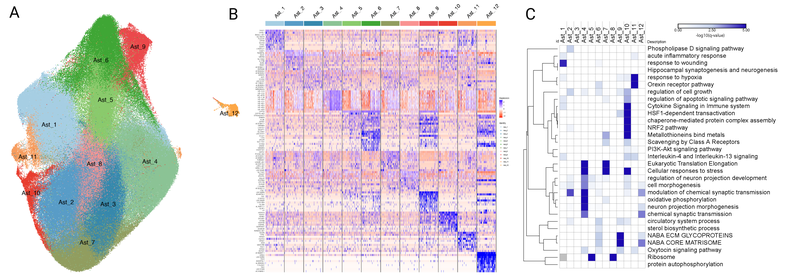


Figure 1: Astrocytes cell states in aged human brains PFC.
A). UMAP showing astrocyte cell states (343107 nuclei) Identified in PFC615 integrated ROSMAP dataset. B). Gene expression of marker genes across cell states. C). Gene ontology/pathway enrichment analysis of top 100 markers genes of microglial cell states.

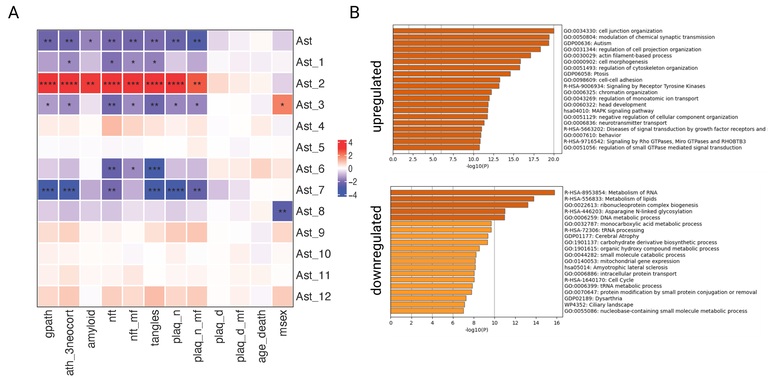


Figure 2: Compositional changes and differential expression analysis of astrocytes in AD
A). Compositional changes of astrocyte cell states in AD . B) Gene ontology enrichment analysis of genes associated with global AD pathology

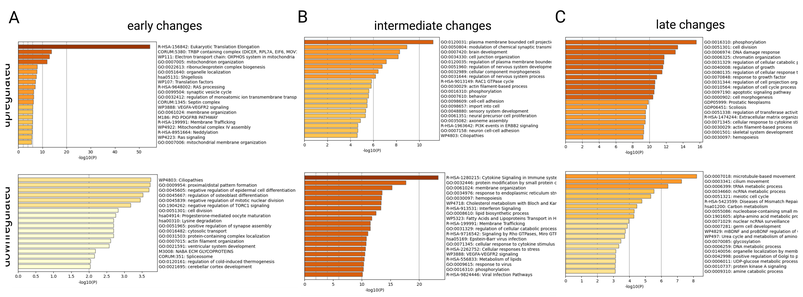


Figure 3: Temporal changes in gene expression across multiple stages of Alzheimer's disease. Gene ontology enrichment analysis of genes associated with global AD pathology in A). early B).intermediate and C). late stages of AD.

RESULTS

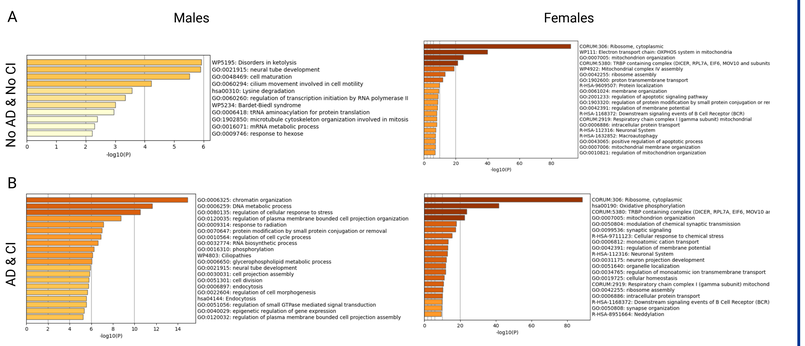


Figure 4: Sex differences in astrocytes.
A). Gene ontology enrichment analysis of **upregulated** differentially expressed genes between **male** and **female individuals** without AD or cognitive impairment (No AD & No CI).
B) Gene ontology enrichment analysis of **upregulated** differentially expressed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

CONCLUSIONS

A number of new transcriptomic changes occurring in **astrocytes** in the **PFC** of **AD patients** were identified. Based on these results, we highlight the following key findings:

- **Astrocyte cell compositions** are **decreased** in AD (at a major celltype level).
- 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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