Review of "Human Microglia Show Unique Transcriptional Changes in Alzheimer's Disease"

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1 Brief Summary

- The paper investigates the transcriptional changes in human microglia associated with Alzheimer's disease (AD). Employing FANS-snRNA-seq, the researchers enriched microglia nuclei from postmortem human brains, identifying both established and previously unrecognized microglial molecular phenotypes and gene networks. They found microglial phenotypes more prevalent in AD cases compared with controls, and described heterogeneity in microglia subclusters expressing homeostatic markers. This study shed light on how microglia gene expression varies in AD, and suggests new avenues for research and potential therapeutic strategies.
- **Question:** What does it mean by saying heterogeneity in microglia subclusters expressing homeostatic markers? What is 'homeostatic' in specific?

2 Objective of the Paper

- To identify clusters with unique transcriptomic profiles, which allows for the discovery of genetic and epigenetic elements that regulate specific cell behaviors.
- To map the full range of microglial transcriptional phenotypes.
- To identify AD-associated gene expression change within a cluster or subcluster.

3 Key Findings

- **Identification of Microglia Clusters:** The study identified 10 distinct microglia clusters, each with unique gene expression patterns and biological pathways.
- **AD-specific Microglia Phenotypes:** A specific microglia phenotype, more prevalent in Alzheimer's disease (AD) cases, was discovered.
- **Transcription Factor Regulatory Networks:** Unique transcription factor regulatory networks for each microglia cluster were revealed.
- **Microglia Transcriptomic Progression:** The research showed potential transitions of microglia to multiple endpoint phenotypes from a 'homeostatic' state in AD.
- **Increased Inflammatory ELN Profile in AD:** An increase in the inflammatory ELN profile in microglia clusters was observed in AD cases.

4 Methodology

- Microglia nuclei were isolated from postmortem human brains using fluorescence-activated nuclei sorting (FANS) for PU.1, a myeloid-specific transcription factor, followed by single-nucleus RNA sequencing.

- Preprocessing procedures were performed that data were normalized for read depth and mitochondrial gene content using Seurat. The analysis focused on the most variable 5,000 genes.

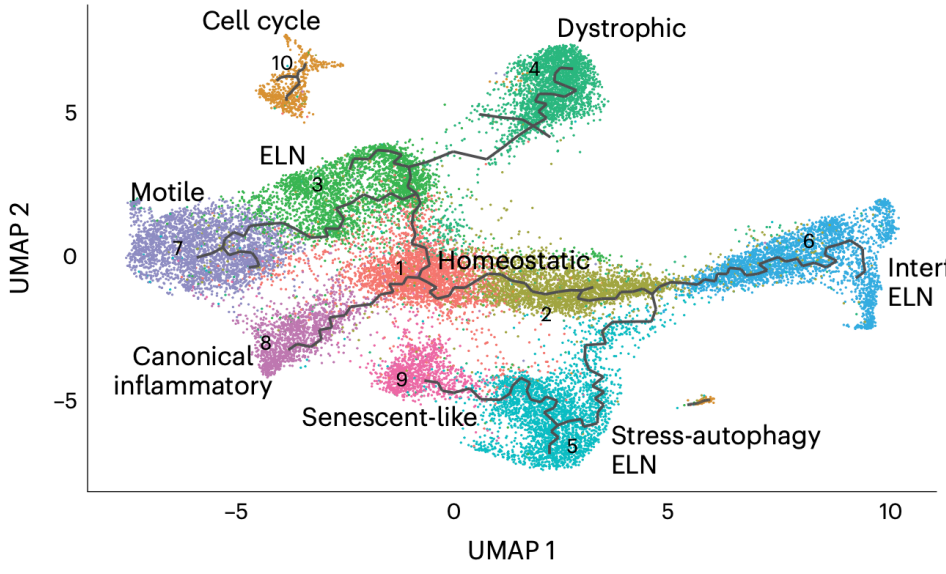
- Using fifteen principal components, a graph was constructed to identify microglia clusters based on nearest neighbors. The Louvain algorithm was applied to define these clusters, each with a set of defining genes.

- Clusters were annotated for cell type using known genetic markers.

- The SCENIC workflow was utilized to infer gene regulatory networks within the identified clusters.

- **Question:** I don't think I need to focus on the details of FANS?

5 Figures



- **Fig.4** What are these?

6 Limitations

- The possibility that PU.1 sorting may selectively enrich certain microglia types
- Possibility of misidentifying other myeloid cell types as microglia
- A limited number of subjects
- The potential discrepancy between gene and protein expression.
- Using postmortem brain tissue might introduce variables affecting the results, and the predominance of an older cohort could skew gene expression interpretations.

7 Aspects of personal interests

- **Machine learning:** I'm interested in the various ML models and algorithms that were employed in this study or in sn-RNA-seq analysis in general. (Seurat packages, PCA, Louvain and Leiden Algorithms, etc.)
- **Clustering & cell identity annotation:** The discovery of distinct transcriptomic profiles in microglia specifically associated with Alzheimer's disease.
- **Novel Genetic and Epigenetic Insights:**Uncovering new genetic and epigenetic factors that regulate microglial behavior in the context of Alzheimer's and how these findings might contribute to the development of precision medicine strategies for Alzheimer's disease.

8 Other questions in general

- Why are we looking at the subclusters?
- Can the identified transcriptomic clusters of microglia be linked to specific stages or severity of Alzheimer's disease?
- How to define biological pathways? What does GSEA do? Am I supposed to now these?
- What are gene regulatory networks (GRNs) and what does the SCENIC workflow in Python do?
- This paper appears to be a collaborative effort of experts in neuroscience (lots of biological terminologies), and biostatistics. I'm wondering what our specific contributions/roles would be in this context.

9 References

- Prater, K.E., Green, K.J., Mamde, S. et al. Human microglia show unique transcriptional changes in Alzheimer's disease. Nat Aging 3, 894–907 (2023). https://doi.org/10.1038/s43587-023-00424-y