Final Project Report

Project Title:

Is Gene Expression in Glial Cells Altered in Autism Spectrum Disorder?

Group Members and Contributions

This project was carried out individually by Wateen Alshawi, who contributed to all stages of the research, including data preprocessing, quality control, differential expression analysis, visualization, and preparation of the final report and poster.

Project Mentors

- Prof. Hugo Tavares, Dr. Chandra Chilamakuri

Project Objectives and Goals

- To analyze single-cell RNA sequencing (scRNA-seq) datasets from postmortem brain samples of individuals with Autism Spectrum Disorder (ASD).
- To identify cell type–specific transcriptional changes, focusing particularly on astrocytes and oligodendrocytes.
- To better understand the molecular and cellular mechanisms contributing to ASD pathophysiology.
- To develop bioinformatics skills through hands-on data analysis and scientific communication.

Current Progress and Milestones Achieved

- Successfully imported and preprocessed ASD scRNA-seq datasets.
- Performed quality control and normalization of expression counts.
- Applied dimensionality reduction (PCA, T-SNE) to visualize cellular heterogeneity.
- Focused analysis on astrocytes and oligodendrocytes for differential gene expression.

- Presented findings in a poster session at the Genetics Department, Cambridge, where the project received recognition as **Best Poster Award**. The award was judged and granted by **Prof. Paul Fannon** (Teaching Associate, Bioinformatics Training Facility, University of Cambridge, recipient of an OBE for services to education) and **Prof. Matt Castle** (Head of the Bioinformatics Training Facility and Teaching Professor of Mathematical Biology & Bioinformatics, University of Cambridge), who were also the principal organizers of the program.

Methodology and Approach

1. Data Acquisition – Publicly available scRNA-seq datasets of ASD and control brain tissues.
2. Preprocessing & Quality Control – Filtering out low-quality cells, removing high mitochondrial content cells, and normalization of gene expression using log-counts.
3. Dimensionality Reduction & Clustering – PCA and T-SNE [Fig.1] were performed.
4. Subset Analysis – Astrocytes and oligodendrocytes were extracted and analyzed in detail.
5. Differential Expression Analysis (DEG) – Conducted using the scran package, producing log fold-change, p-values, and FDR-adjusted significance values.
6. Visualization – Heatmap [Fig.2], volcano plots, and cluster embeddings were generated to display transcriptional patterns and highlight significant differences between ASD and controls.
7. Pathway Enrichment – Applied KEGG and Gene Ontology (GO) analysis on differentially expressed genes to explore dysregulated biological pathways in ASD.

Preliminary Results or Findings

- Astrocytes: Upregulation of immune response–related genes, suggesting altered inflammatory signaling in ASD.
- Oligodendrocytes: Downregulation of myelin-associated genes, indicating impaired neuronal insulation and connectivity.
- Alterd Gene XIST gene which is related to the X chromosome expression
- Overall, the findings support a cell type–specific vulnerability model of ASD, in which glial cells play a critical role in shaping disease pathology.

Challenges and Obstacles Encountered

- Computational intensity of handling large-scale single-cell data..
- Time constraints for analysis and presentation preparation.

Next Steps and Future Work

- Expand the analysis to additional brain regions (e.g., prefrontal cortex).
- Perform pathway enrichment analysis to identify converging biological mechanisms.
- Investigate cell–cell communication patterns using dedicated tools (e.g., CellChat, NicheNet).
- Cross-validate results with larger ASD datasets and integrate other omics layers (e.g., epigenomics, proteomics).
- Subset the data by gender to investigate differences in XIST gene expression, which may provide insight into sex-specific transcriptional regulation in ASD.

Resources and Tools Used

- Datasets: Public scRNA-seq datasets of ASD and control brain samples. Velmeshev D, Schirmer L, Jung D, et al. Single-cell genomics identifies cell type–specific molecular changes in autism. Science. 2019;364(6441):685–689. doi:10.1126/science.aav8130
- Software & Packages: R (scater, scran, ggplot2), T-SNE, PCA tools.
- Computational Tools: RStudio, Linux environment.
- Code Repository: https://github.com/wateenalshawi-max/SingleCell_RNASeq_AnalysisGlialCells.git
- References: Recent single-cell ASD studies, methodological bioinformatics papers.

Acknowledgments

Special thanks to Prof. Hugo Tavares, Dr. Andries, Prof. Paul Fannon, Prof. Matt Castle, and Dr. Chandra for their mentorship and invaluable support throughout the project. My friends Rahaf Kassar and Shaden Almohawwes for their support and help throughout the journey.

Fig.1 T-SNE of Cell Types Distribution

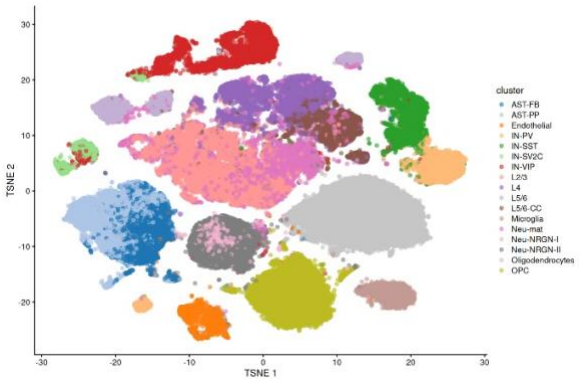


Fig.2. Heatmap of DEGs Across Cells

