Microglial Single Cell Transcriptomics Reveal Drivers of Autism Severity

Background

Autism Spectrum Disorder (ASD)

- Prevalent neurodevelopment disorder characterized by repetitive behaviors and challenges with communication and social interactions
  - Severe disease involves intellectual disability and epilepsy (recurrent seizures)
- Despite clinical differences, brain anatomy and tissue structures similar to normal brain
  - DNA mutations present in about 20% of cases, however with limited overlap across patients
  - Reproducible changes in RNA expression previously documented in ASD patients in numerous cell types, including microglia

Microglia Single Cell RNA Sequencing

- Microglia are brain resident immune cells responsible for immune surveillance and neurodevelopment
- Dysregulated microglial activation can drive neuroinflammation and disease
- Microglial gene expression profiles offer an unbiased view of cell states during disease and can be studied at the single cell level with publicly available RNA sequencing (scRNA Seq) data from previous ASD publications

Project Aims

Problem

- ASD shows marked clinical heterogeneity in severity, yet the molecular mechanisms underlying disease severity remain poorly understood
- While immune dysregulation has been implicated in ASD, it is unknown which microglial gene programs drive disease severity.

Question

- *How do microglia-specific gene expression pathways change across increasing ASD severity?*

Hypothesis

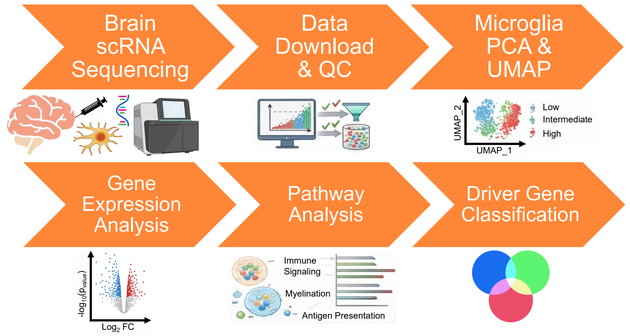
- Given that microglia are involved in ASD development, we hypothesize that they will demonstrate significant differences in multiple gene expression programs that evolve across low, intermediate, and high severity disease states.

Materials

- Source Data: Wamsely et al., Molecular cascades and cell type-specific signatures in ASD revealed by single-cell genomics. *Science* 384, eadh2602 (2024)
  - Processed post mortem pre-frontal cortex single cell RNA sequencing data from 33 ASD patients
  - 35 identified cell types → microglia focus
- Computer with 64 GB RAM
- R Studio & Programs
  - Seurat scRNA Seq Bioinformatics Toolkit
  - Ggplot2 Data Visualization Library
  - Gene Ontology Pathway ClusterProfiler Library

Methods

Overview



Detailed Methods & Statistical Analysis

1. UCSC Cell Browser Data Download & Quality Control
   - Cells filtered to remove low-quality cells based on gene counts, and mitochondrial gene expression
   - Pre-annotated Microglia cells were exported
2. UMAP Dimensionality Reduction
   - Principal component analysis (PCA) followed by UMAP was applied to visualize microglial transcriptional states.
3. Differential Gene Expression Analysis
   - Significant Genes: Benjamini-Hochberg adjusted p-value < 0.01 and $|\log_2$ fold change$| > 2.5$
   - Low vs Intermediate
   - Intermediate vs High
   - Low vs High
4. Gene Ontology (GO) Pathway Analysis
   - Significant genes enriched with GO Biological Process enrichment to identify functional pathways
5. Driver Gene Classification
   - Early drivers: significant only in Low vs Intermediate
   - Late drivers: significant only in Intermediate vs High
   - Cumulative drivers: significant only in Low vs High
   - Global drivers: significant across all comparisons

See Supplementary Methods for full details and code

Results

Table 1: ASD Patient Cohorts

De-Identified Patient ID	Age	Gender	Cause of Death	Clinical Symptoms	Disease Severity
KMC3	48	Male	Natural	Cancer, Gastric carcinoma	Hyperkinesis, Pica	Low
2NA6	16	Male	Accident	Blunt force trauma	Bipolar	
493	26	Male	Accident	Drowning	Blind	
FXMW	29	Male	Natural	Cardiac Arrest	ADHD	
5023	16	Male	Accident	Blunt force trauma	Epilepsy, Diabetes	Intermediate
9714	60	Male	Natural	Cancer, Pancreatic	Epilepsy	
19511	8	Male	Natural	Cancer (Sarcoma)	Epilepsy	
5302	16	Male	Natural	Diabetic Ketoacidosis	Epilepsy	
6041	19	Male	Natural	Seizure	Epilepsy	
8792	29	Male	Natural	Acute pancreatitis-Renal Failure	Epilepsy	
12457	29	Female	Natural	Seizure	Epilepsy	
2YK7	17	Female	Natural	NA	Intellectual Disability, Epilepsy	High
3HUF	23	Male	Natural	Pneumonia	Intellectual Disability, Epilepsy	
VPSP	20	Male	Natural	NA	Intellectual Disability, Epilepsy, ADHD	
8XCF	27	Male	Natural	Acute pancreatitis-Renal Failure	Intellectual Disability, Epilepsy	
M9H3	59	Female	Natural	Seizure, Cardiac Arrest	Intellectual Disability, Epilepsy	
5842	19	Male	Natural	Cardiac Arrest	Intellectual Disability, Epilepsy	
13161	24	Male	Natural	NA	Intellectual Disability, Epilepsy, ADHD	

- Inclusion Criteria: Diagnosis of ASD by DSM V,
  - Low Severity: No Epilepsy or Intellectual Disability Clinical Symptoms
  - Intermediate Severity: Epilepsy (short term impairment) without Intellectual Disability (long term impairment)
  - High Severity: Both Intellectual Disability and Epilepsy
- Exclusion Criteria
  - 15q Duplication Genetic Subtype n=5 (confounding molecular driver of disease)
  - Unreported Clinical Symptoms n=10 (unclear severity)

Figure 1: Microglia Transcriptional Landscape Across ASD Severity

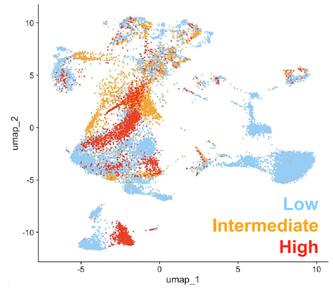
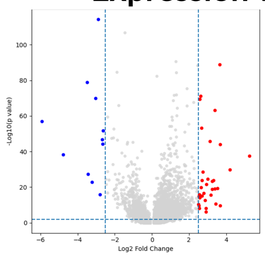


Fig. 1 Legend: UMAP projection of microglia transcriptional profiles across ASD severity cohorts. Each dot represents a unique cell. Cells from each cohort occupy shared and distinct transcriptional clusters, suggesting a progressive alteration in global cell states.

Figure 2: Differential Microglial Gene Expression with ASD Severity



Comparison	Significant Genes	Up-regulated	Down-regulated
1) Low vs Intermediate	53	45	8
2) Intermediate vs High	43	32	11
3) Low vs High	135	129	6

Fig. 2 Legend: Volcano plot of significantly upregulated (red) or downregulated (blue) genes (individual dots) between intermediate and high severity ASD microglia. Table summarizes results across all three comparisons

Figure 3: Microglial Activation & Stress Responses Associate with ASD Severity

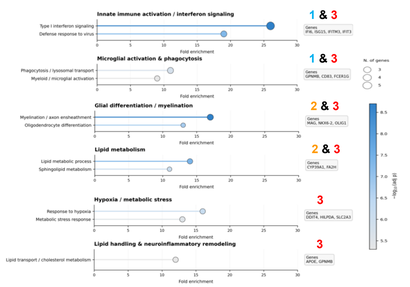
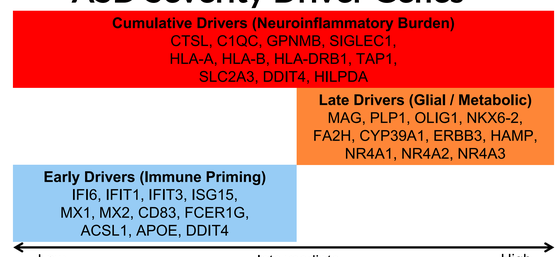


Fig. 3 Legend: GO Pathway enrichment analysis of differentially expressed genes across severity comparisons (1-3). Biological processes grouped by functional theme. Pathway gene fold enrichment plotted along with heat map of adjusted p value and number of involved genes (circle size)

Results (continued)

Figure 4: Candidate Microglia ASD Severity Driver Genes



Type	Significant Genes
Early Driver	12
Late Driver	30
Cumulative Driver	90
Global Driver	0

Fig. 4 Legend: Summary classification of significant, non-overlapping genes across each stage of ASD severity. Disease severity associated with distinct drivers on a shared continuum.

Summary

Conclusions

- ASD severity-associated transcriptional changes in microglia follow a trajectory involving early immune priming, later glial/metabolic remodeling, and cumulative neuroinflammatory burden
- Lack of global drivers suggests distinct microglial molecular states associated with disease severity

Limitations

- Severity cohorts are based on broad clinical symptoms, which are likely different across patients
- Differential expression analyses is correlative and does not establish definitive causal relationships
- Conclusions are based on microglia data alone, key interactions with excitatory neurons and other brain cells to be explored

Future Directions

- Evaluate a machine learning model of disease severity and compare with current results
- Validate results in independent data sets
- Evaluate functional consequences of targeting driver genes in microglia in ASD disease models

Bibliography

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