Exploring the cellular and temporal specificity of neuological disorder risk genes in human brain development

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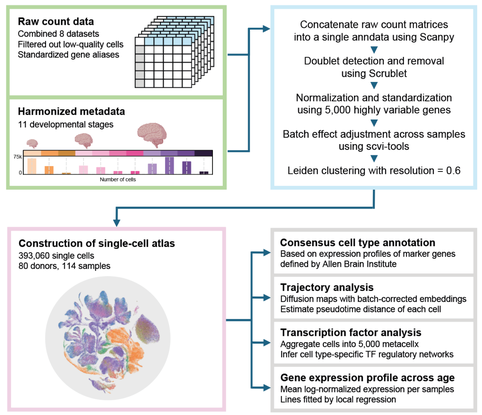
ABSTRACT

Advancements in single-cell technologies have transformed transcriptomic studies across brain regions, enhancing our understanding of the human brain. However, pinpointing cell-type specificity in neurological disorders remains challenging due to developmental variations. To address this, we analyzed neurological disorder gene expression dynamics using a single-cell transcriptome dataset spanning multiple developmental stages. Our atlas, comprising 393,060 cells and nuclei, reveals distinct temporal expression patterns of disorder risk genes, including autism, across neuronal lineages. We identified a concentration of neurological disease traits within fetal cell types, providing insights into the dynamic regulation of risk genes during brain development. This study offers a foundation for comparing cell type-disorder associations over time, advancing our understanding of neurological diseases.

METHODS



Schematic of atlas construction and downstream analysis. Single-cell and single-nucleus RNA sequencing datasets from eight published studies were integrated. After applying quality control measures, gene name standardization, and batch effect correction, cells were clustered using the Leiden algorithm. Clusters were annotated based on developmental stages and brain regions, and major cell types were identified using marker genes. Downstream analyses involved trajectory analysis and the inference of gene regulatory networks.

RESULTS

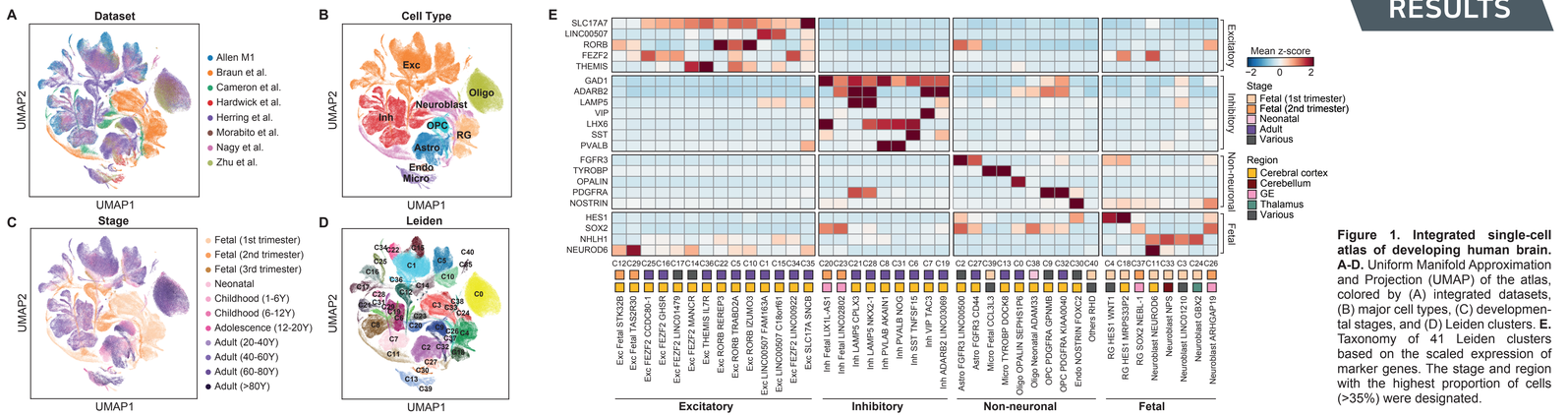


Figure 1. Integrated single-cell atlas of developing human brain. A-D. Uniform Manifold Approximation and Projection (UMAP) of the atlas, colored by (A) integrated datasets, (B) major cell types, (C) developmental stages, and (D) Leiden clusters. **E.** Taxonomy of 41 Leiden clusters based on the scaled expression of marker genes. The stage and region with the highest proportion of cells (>35%) were designated.

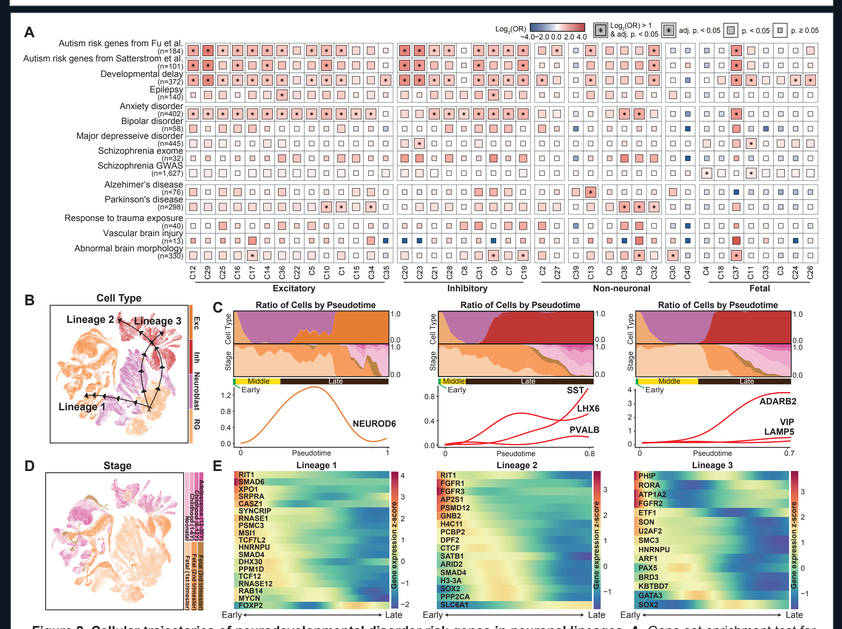


Figure 2. Cellular trajectories of neurodevelopmental disorder risk genes in neuronal lineages. A. Gene set enrichment test for neurological disorder genes. A one-sided Fisher's exact test was used to compute statistics with multiple comparisons by Bonferroni correction. **B.** UMAP visualizations of estimated developmental lineages in neuronal cell types. **C.** Distribution of cells by major cell type and developmental stage across pseudo-time and temporal patterns of late neuronal IPC marker (NEUROD6), MGE-derived inhibitory neuron markers (SST, LHX6, and PVALB), and CGE-derived inhibitory neuron markers (ADARB2, VIP, and LAMP5). Cells in the first one-third portion of cells with pseudo-time close to 0 are labeled as "Early", the subsequent portion as "Middle", and the final one-third of cells with the latest pseudo-time are designated as "Late". **D.** UMAP visualizations of developmental stages in neuronal cell types. **E.** Expression profiles of neurodevelopmental disorder risk genes across pseudo-time for each lineage.

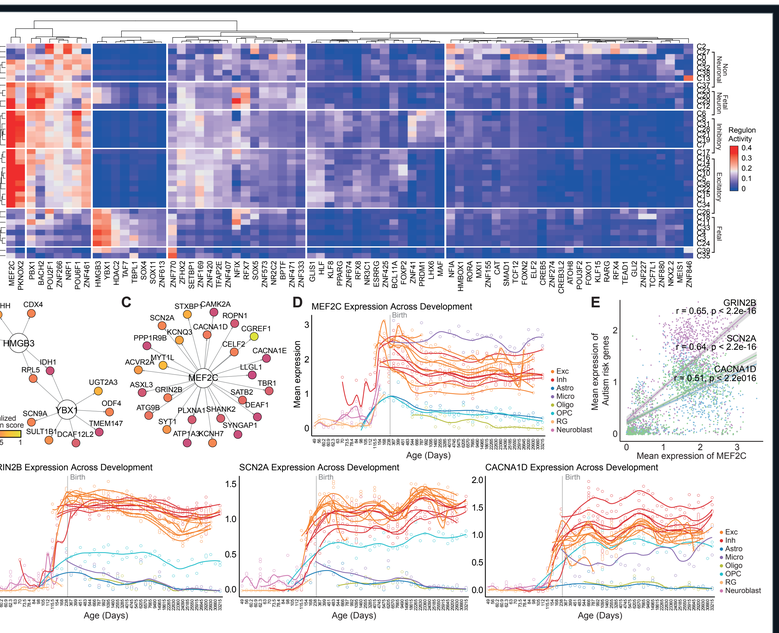


Figure 3. Regulatory landscape in early brain development. A. Heatmap illustrating regulon activities of transcription factors across clusters. **B-C.** Transcription factor-target networks depicting regulation of glioblastoma risk genes by HMGB3 and YBX1 (B), and regulation of autism risk genes by MEF2C (C). Prediction confidence was normalized from 0 to 1. The top 25 targets high-confidence targets for MEF2C are shown. **D.** Expression of MEF2C over gestational days. The sample-wise mean of log-normalized MEF2C expression was computed using a pseudo-bulk method. Clusters with at least 4,600 cells (C0-C22) were used. **E.** Correlation between the sample-wise mean of log-normalized MEF2C expression and expression of GRIN2B, SCN2A, andCACNA1D. **F.** Expression of GRIN2B, SCN2A, and CACNA1D across developmental ages.

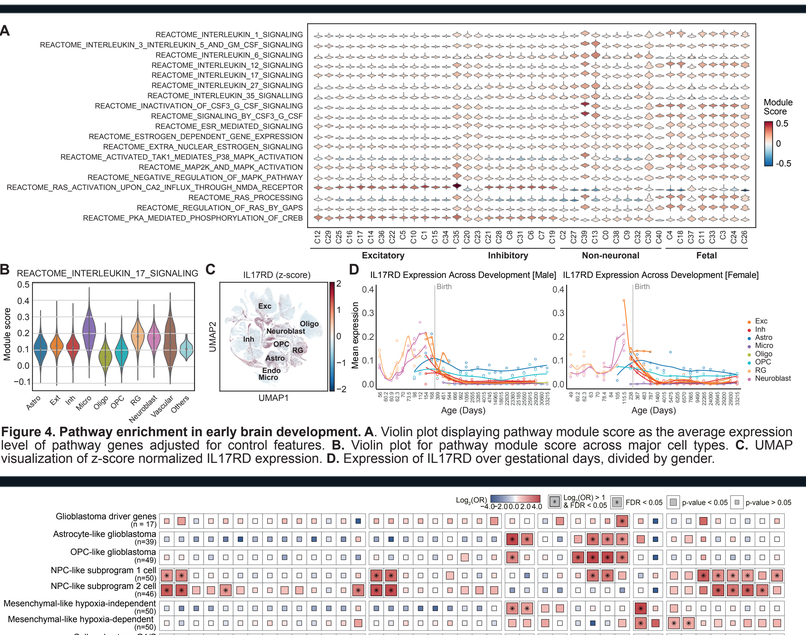


Figure 4. Pathway enrichment in early brain development. A. Violin plot displaying pathway module score as the average expression level of pathway genes adjusted for control features. **B.** Violin plot for pathway module score across major cell types. **C.** UMAP visualization of z-score normalized IL17RD expression. **D.** Expression of IL17RD over gestational days, divided by gender.

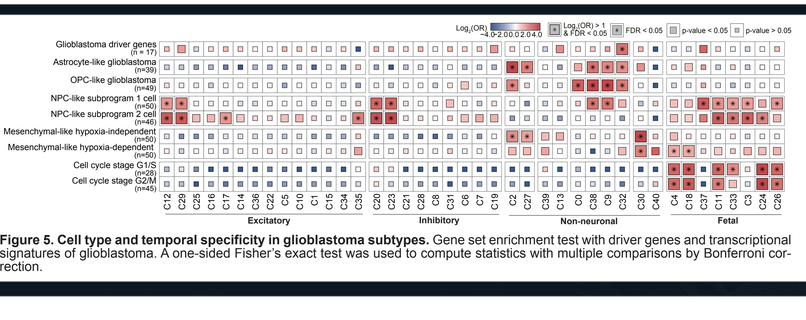


Figure 5. Cell type and temporal specificity in glioblastoma subtypes. Gene set enrichment test with driver genes and transcriptional signatures of glioblastoma. A one-sided Fisher's exact test was used to compute statistics with multiple comparisons by Bonferroni correction.

DISCUSSION

- Constructed a single-cell atlas of the developing human brain, analyzing 393,060 single brain cells to reveal the cellular composition and dynamic changes during early brain development, focusing on the cellular and temporal specificity of neurological disorder risk genes.
- Revealed distinct expression patterns of autism risk genes (e.g., FOXP2) at different developmental stages. Also identified the role of MEF2C in aligning expression patterns with autism risk genes and highlighted potential sex differences in IL17RD expression related to MIA susceptibility.
- This study faced limitations such as incomplete representation of individual variability and developmental stages, particularly neonatal and early childhood periods. Future research should aim for a more extensive and diverse sample collection to better understand the genetic constitution's role in early brain development and its impact on disorders.

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