Identification of cell-types and biological pathways associated with autism spectrum disorder using single-cell genomic analysis

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Autism Spectrum Disorder (ASD) has been largely associated with genetic abnormalities through studies with bulk genomic data. Single-cell sequencing technology enabled the identification of associated cell-types and differentially expressed genes. This project aims to identify relevant cell-types in ASD versus control subjects, as well as differentially expressed and differentially methylated genes at the single-cell level through multi-omic analysis (snmCT-seq). Although the effect size of individual genes was small, Pathway over-enrichment analysis indicates the involvement of excitatory (IT-L2 – IT-L6), inhibitory (MGE-Pvalb, MGE-SST, CGE-Vip, Sncg), and non-neuronal (ASC, ODC/OPC) cell-types in several disorder-relevant pathways, such as the reduction of cytosolic Ca2+ levels (ATP2B1, SLC8A1, CALM1) in RNA and cation-coupled chloride cotransporters (SLC12A3, SLC12A5) in methylation. These omic pathway differences could have behavioral implications given the role of calcium in neurotransmitter release and membrane excitability, leading to the cognitive differences observed in ASD patients.

Table 1	Control	ASD
Participants	12	12
Age	26.33 ± 17.7	20.33 ± 12.1
Race	25% White 33.33% Black 41.66% NA	16.66% White 8.33% Black 75% NA

Identifying Cell Types

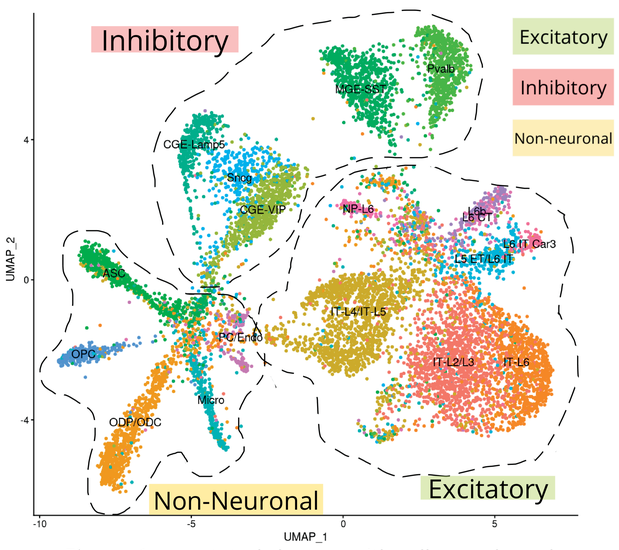


Figure 1: annotated clusters with cell types based on expression of marker genes

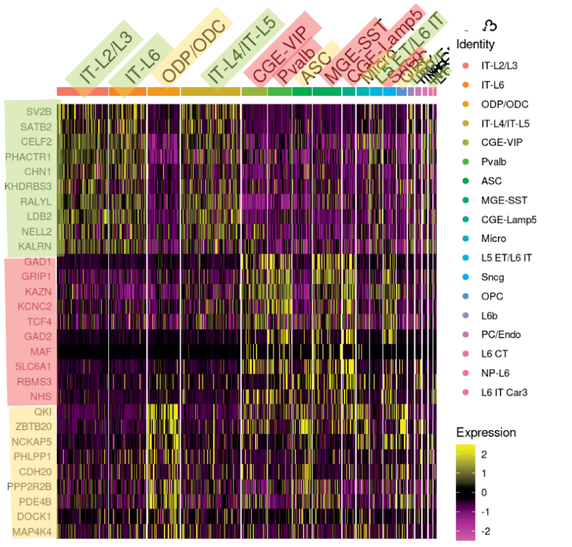
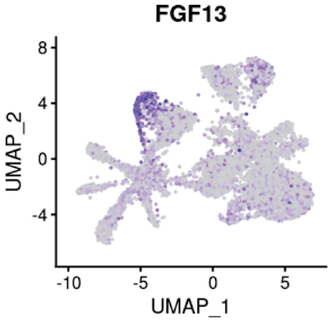


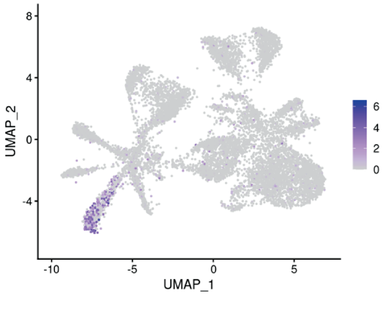
Figure 2: heat map verifying the classification of cell types using known marker genes

Table 2 Cell Type	Cell Count
IT-L2/L3	1526
IT-L6	1114
ODP/ODC	962
IT-L4/L5	1781
CGE-VIP	756
Pvalb	705
ASC	568
MGE-SST	855
CGE-Lamp5	383
Microglia	382
L5 ET/L6 IT	367
Sncg	367
OPC	303
L6b	191
PC/Endo	186
L6 CT	152
NP-L6	123
L6 IT Car3	99
Sum	10820

CGE-Lamp5



ODP/ODC

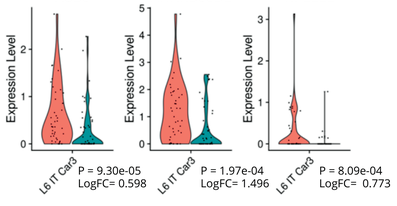


Figures 3 & 4: marker genes used to classify cell types based on localized expression

The expression of genes associated with certain cell types—known as marker genes—should be relatively localized to a certain cluster. Annotations of each cluster can be made based off of these genes.

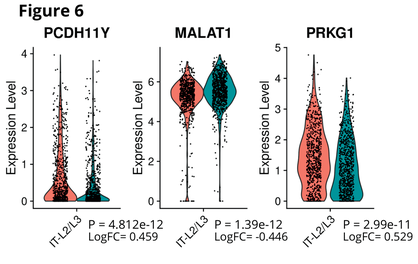
L6 IT Car3

Figure 5



IT-L2/IT-L3

Figure 6



= ASD

= Control

LogFC > 0 means greater ASD
LogFC < 0 means greater CTL

Figures 5 & 6: Differentially expressed genes for each cell type were extrapolated and visualized for RNA and methylation.

Figures 8, 9, 10, & 11: These genes were then used to find relevant biological pathways in RNA and methylation that may contribute to the ASD phenotype.

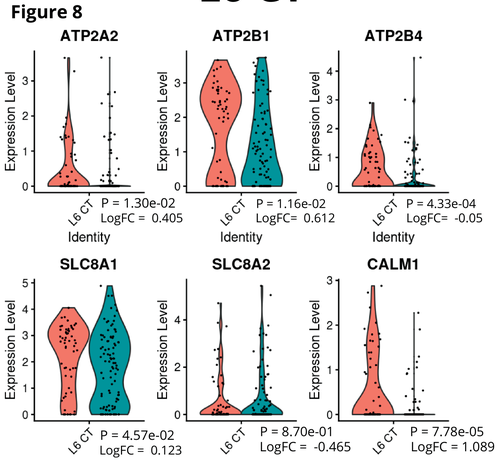
Associated Pathways

Figure 7

RNA	Methylation
Reduction of Cytosolic Ca++ P < 0.0005	**Increased cation-coupled chloride cotransporters** P < 0.005
↓	↓
Altered neurotransmitter release & membrane potentiation	**Altered ionic concentration**
↓	↓
Cognitive Differences	**Cognitive Differences**

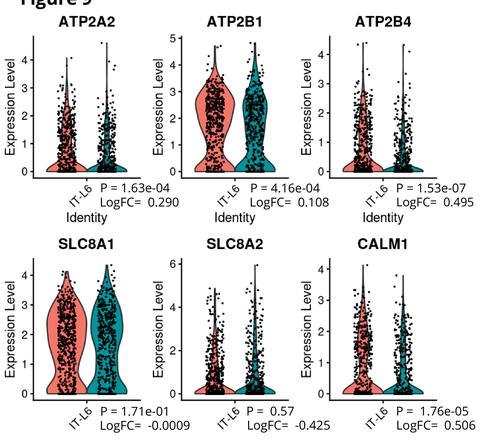
L6 CT

Figure 8



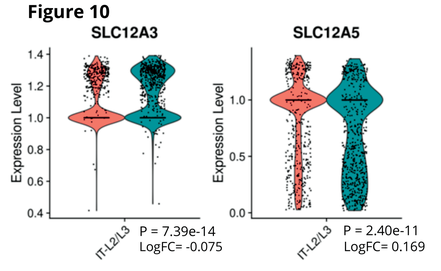
IT-L6

Figure 9



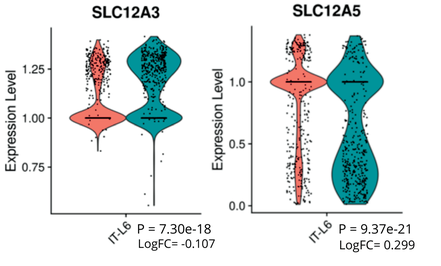
IT-L2/L3

Figure 10



IT-L6

Figure 11



Discussion

Pathways involving Calcium and Chloride ions were found to be significant in RNA and methylation, respectively. Disrupting the function of these pathways may alter cell homeostasis, leading to some of the cognitive differences seen in ASD patients. Next steps involve comparing these results to known SNPs associated with ASD to look for overlap and new findings. Understanding the role of these pathways and related SNPs could lead to viable future treatments.