Spatial Transcriptomic Analysis in Alzheimer's Disease

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Introduction

- Spatial transcriptomics is an RNA sequencing technique which preserves spatial information
- Alzheimer's (AD) is a neurodegenerative disease categorized by degradation in memory and processing skills, marked by an increase in amyloid-beta plaques and neurofibrillary tangles. It is associated with axon loss in white matter, as well as neuronal death and inflammatory response of grey matter
- Analysis of the genetic co-occurrence of Down Syndrome (DS) and AD is of importance because individuals with DS have an earlier onset age and a higher prevalence of AD
- Differentially expressed genes (DEGs) are genes that have significantly different expression levels between two groups
- Spatially variable genes (SVGs) are genes whose expression levels are dependent on location

Methods

Data and Exploratory Analysis

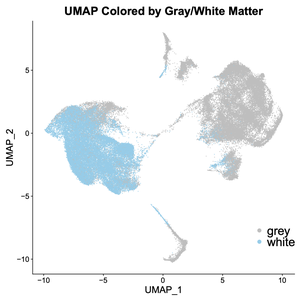


Table 1. Summary Demographics

	Total Count	Median Age	Count by Sex	
			Male	Female
Control	10	76.5	5	5
Early AD	9	87.0	6	3
Late AD	10	89.5	4	6
AD w. DS	10	56.5	3	7

Spots	x	y	Annotations
S_1	x_1	y_1	z_1
S_2	x_2	y_2	z_2

DEGs - edgeR

- Utilized a negative binomial distribution to fit gene expression count data (edgeR), collapsed by summing across spots within each sample[4]
- Controlled for false discovery rate (FDR) set to 0.1 using Benjamini Hochberg adjustment

SVGs – Spark-X

- Utilized a non-parametric covariance-based approach (Spark-X) to derive gene-specific p-values for each sample[5]
- Combined p-values across disease stages using the Cauchy combination method

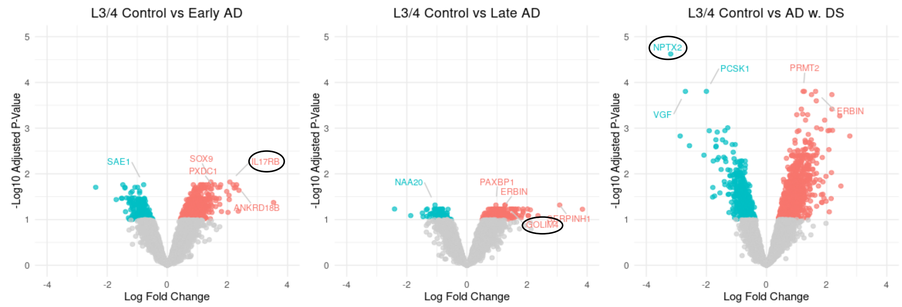
Gene Set Enrichment Analysis (GSEA)

- Performed GSEA (fgsea) to determine whether predefined gene groups (e.g. pathways) are significantly enriched at the top or bottom of a ranked list of DEGs[2]
- Used over-representation analysis (gprofiler2) to determine biological pathways significantly enriched in SVGs[1]

DEG Results

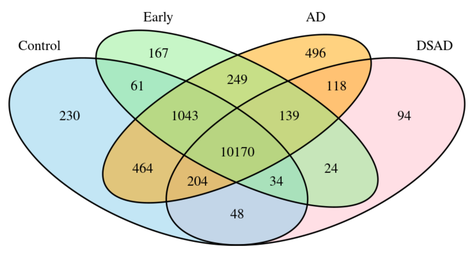
Table 2. DEG Counts (FDR=0.1)

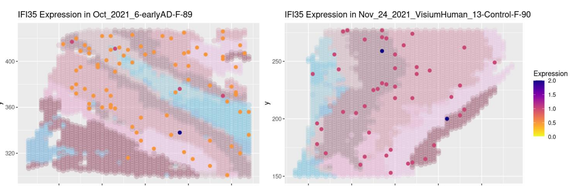
	Bulk	White Matter Regions				Grey Matter Regions						
		All	*WM1*	*WM2*	*WM3*	*All*	*L1*	*L2/3*	*L3/4*	*L3/4/5*	*L5/6*	*L6b*
Ctrl v Early	0	0	0	1	0	0	5	12	743	136	0	0
Crtl v Late	0	0	0	0	0	0	3	0	329	0	0	0
Crtl v DS	1574	2	11	0	0	2993	109	449	1637	1177	515	15



Volcano plots of L3/4 DEGs. FDR set to 0.1. Upregulated genes (in disease group) in red, downregulated in blue. Top five significant genes labelled with abs(log(FC)) > 0.25 and circled genes further analyzed.

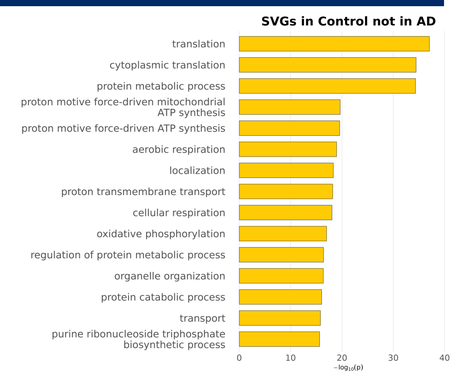
SVG Results





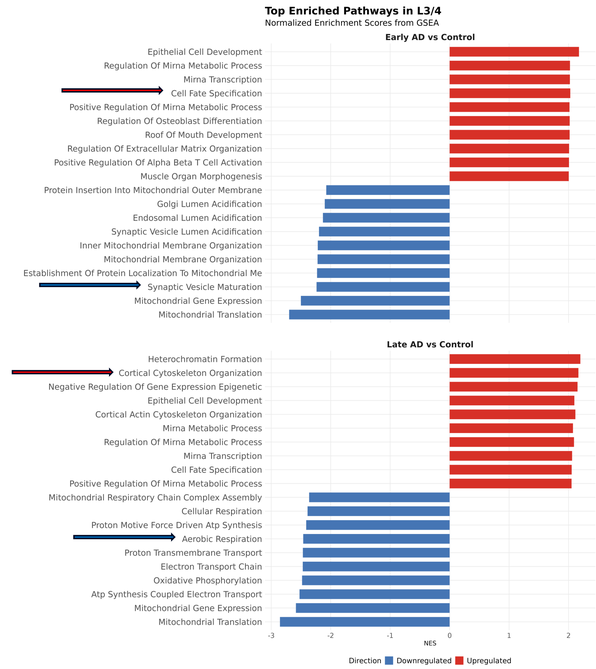
- IFI35 is linked to inflammation signaling

- APLP1 is linked to neurogenesis



Over-representation analysis of SVGs in late AD.

DEG GSEA Results



Enriched pathways in grey matter L3/4. Pathways in mitochondrial dysfunction, cytoskeletal organization, and cell fate up- or down-regulated.

Discussion

- DEGs were found primarily in grey matter, and the corresponding upregulated pathways relate to inflammatory response and neurogenesis, and downregulated pathways relate to mitochondrial dysfunction
- SVG analysis primarily found genes linked to inflammation signalling, neuronal death, and cell cycle arrest
- All of these are postmortem samples and the time to sequencing from death is inconsistent, leading to different amounts of tissue decay
- Across all groups we had small sample sizes impacting the power of our analysis, potentially leading to low signal in the dataset

Acknowledgements

Thank you to Dr. Matt Zawistowski, Peiyao Zhao, Zheng Li, Hanna Venera, Neo Kok, Sabrina Olsson, the 2025 BDSI Genomics Research Group, and all BDSI staff and fellow participants

References

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