Cell-specific Gene Regulatory Networks in Alzheimer's Disease: A Differential Analysis Across Cell Types

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Background

- Alzheimer's disease (AD): most common cause of dementia; how regulation fails inside individual cells is still unclear
- Neurons and microglia are affected differently; AD alters gene *regulation*, not only expression level
- **Gene regulatory network (GRN):** transcription factors (TFs) regulate target genes; edges weighted by regulatory strength
- Paired single-cell sequencing (scRNA-seq for expression + scATAC-seq for chromatin accessibility) enables *one GRN per cell*

Research question

Compare cell-specific GRNs across AD severity to find which regulatory changes occur, and whether they are shared between cell types or specific to one.

- **Edge level:** does each TF→target link shift with severity?
- **Module preservation:** is the co-regulation structure retained at higher severity?
- **Module activity:** does a module's overall activity change with severity?

Why one network per cell?

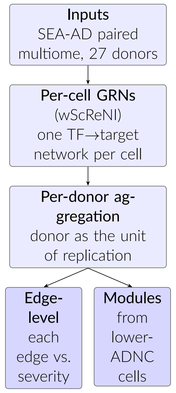
- Cells of one type still differ in active genes and open chromatin
- One network per cell type averages over this and can hide subset-specific changes
- Per-cell networks localise where regulation is disrupted

Dataset: SEA-AD MTG multiome

- SEA-AD middle temporal gyrus (MTG) [2]: paired snRNA-seq + snATAC-seq, same nuclei
- Severity graded by Overall AD Neuropathological Change (**ADNC**): four ordinal levels (Not AD, Low, Intermediate, High)
- Reference contrast: **lower-ADNC** (Not AD/Low) vs **higher-ADNC** (Intermediate/High)
- Observational cohort, graded by severity (not case/control)
- Two subclasses examined: **L2/3 IT** (excitatory neurons), **Microglia-PVM** (resident immune population)

Method and pipeline

- **wScReNI** [1]: per cell, pool its nearest neighbours (k-NN), train a random forest per target gene; feature importances become edge weights
- Reimplemented from R into Python and applied to AD data



Statistical design

- Per-cell networks averaged within donor and cell type; **donor = unit of replication** (27 donors: 7 lower-ADNC, 20 higher-ADNC)
- Each edge/module regressed on ordinal ADNC severity, controlling for age, sex, and two co-pathologies: LATE (TDP-43 proteinopathy) and LBD (Lewy body disease)

$$\text{weight} \sim \text{ADNC} + \text{age} + \text{sex} + \text{LATE} + \text{LBD}$$

- Significance at Benjamini–Hochberg false discovery rate (FDR) $q < 0.05$

Edge level: no robust differential edges

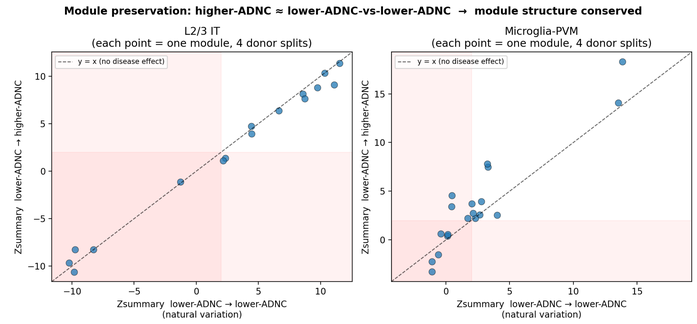
- Mean-weight filtering separates a true absence of signal from the multiple-testing cost
- All detected edges **weaken** with severity

Edge	$\log_2$FC	q at filter strength				
		0%	25%	50%	75%	90%
L2/3 IT						
LEF1→HEG1	−2.31	0.050	0.045	0.038	filt.	filt.
E2F2→ADCY4	−2.86	0.050	0.045	filt.	filt.	filt.
ZNF581→DAB1	−0.94	0.050	0.045	0.045	filt.	filt.
Microglia-PVM						
JUNB→NKAIN2	−0.64	0.079	0.062	0.041	0.036	0.015
CEBPD→CYP27C1	−1.06	0.079	0.062	0.041	filt.	filt.

$\log_2$FC = log-2 ratio of mean edge weight, higher- over lower-ADNC (negative = weaker). q after dropping the lowest

Co-regulation modules are conserved

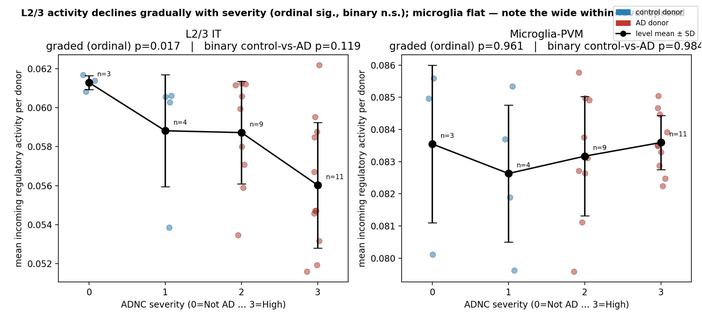
- Modules defined from lower-ADNC cells; preservation tested in higher-ADNC against a matched lower-ADNC baseline (donor-disjoint split, common cell count)
- Between-condition adjacency correlation: $r = 0.86$ (L2/3 IT), $r = 0.70$ (microglia)



Points lie on the identity baseline: modules are preserved at higher ADNC as well as in a matched lower-severity reference.

A global decline in neuronal regulatory activity

- L2/3 IT: every module coefficient negative; 10 of 11 reach $q < 0.05$
- Adding the donor's total activity as a covariate removes all significance: a single **global** decline, not module-specific
- Absent in Microglia-PVM



L2/3 IT severity coefficients are all negative, but significance collapses once global activity is controlled: one global shift, no module-specific effect.

Conclusion

- Networks change relatively little with AD severity in both cell types: no robust differential edge, conserved modules, no module-specific activity shift
- One detectable signal: a modest, severity-graded decline in overall L2/3 IT activity (global, not focal; absent in microglia)
- Next steps: larger cohorts, broader gene panels, more cell types