The Geography of Gene Regulation in Alzheimer's Disease

Inferring and Analysing Spatial Gene Regulatory Networks with ScReNI and Tangram

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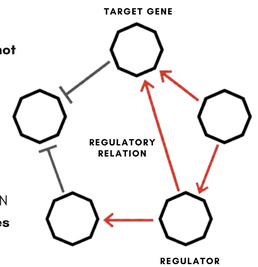
INTRODUCTION AND BIOLOGICAL BACKGROUND

- **Alzheimer's Disease** or **AD** is the most common form of dementia, representing 60% to 80% of all cases [1].
- **Spatial variation** relates to AD, as recent single-cell and spatial transcriptomic studies show that AD does **not develop uniformly across brain tissue**. Instead, disease-associated molecular changes **vary across brain regions** and **cell types** [2].

- A **Gene Regulatory Network** or **GRN** is a system of interacting molecules (genes, proteins, and RNA) that collectively control gene expression within a cell. A GRN can be represented as a **graph**, where **nodes → genes** and **edges → inferred regulatory relationships**.
- **ScReNI** is a recently introduced single-cell regulatory network inference algorithm, which integrates unpaired multimodal data (**scRNA-seq** and **scATAC-seq**) to infer cell-specific GRNs [3].
- **scRNA-seq** data shows gene expression information.
- **scATAC-seq** data indicates which parts of the genome are potentially available for regulation.



EXAMPLE OF A GENE REGULATORY NETWORK

MAIN RESEARCH QUESTION

> Can changes in the spatial location of human brain cells in Alzheimer's disease be related to changes in gene regulatory network structure and the activity of key transcription factors?

THE DATA AND RELATED LITERATURE

- Existing Spatial GRN analysis tools such as SpaGRN [4], SCING [5], and SCRIPro [6] already exist. Each is limited either by its working resolution (cell-cluster or cell-type level) or by assuming that spatially close cells share similar regulation.
- **Tangram** [7] is a computational method that uses overlapping gene expression to optimally **assign each dissociated single-cell** transcriptome to **the most likely spatial spot** and, if desired, impute the full transcriptome at those locations.
- SEA-AD Human MTG 10x paired snRNA-seq + scATAC-seq (1.4M cells) [8] – Allen Institute for Brain Science.
- SEA-AD Human MTG MERFISH (140-gene panel, 1.8M cells) [9] datasets – Allen Institute for Brain Science.

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5. Littman et al. SCING: inference of robust, interpretable gene regulatory networks from single-cell and spatial transcriptomics. iScience, 2023.
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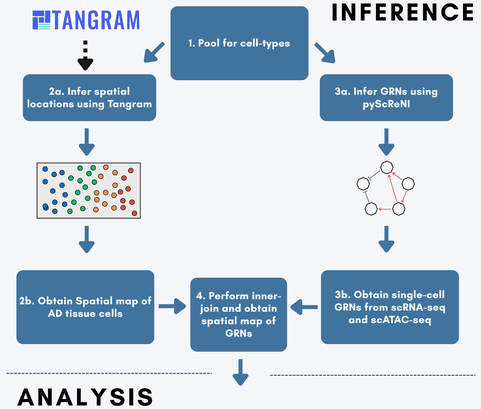
10. Gabitto et al. Integrated multimodal cell atlas of Alzheimer's disease. Nature Neuroscience, 2024.

METHODOLOGY



ANALYSIS

5. Validate Inference

Held out 5% of cells per section and re-mapped them with Tangram, measuring the distance to each cell's true MERFISH location. 22 to 29% landed within 500 µm.

6. Relate Space to GRNs

Tested whether spatially close cells share GRN states, using a variogram, Moran's I on the GRN principal components, and spatial clustering scored by neighbour-purity.

7. Relate Space+GRNs to AD

Correlated the per-donor spatial autocorrelation of GRN components with disease severity (CPS) [10] using a Spearman correlation, with Benjamini-Hochberg correction.

8. Investigate specific regulators

Ranked regulators by the spatial autocorrelation of their target edges, then split each spatial difference into magnitude and shape. Compared individual regulators against global gradient.

GRN inference with pyScReNI

pyScReNI is a functionally equivalent Python port of ScReNI. Developed collaboratively with Eduard Cîmpean, Mihnea Guşu, Ivo Harşani, and Leo Lin.
https://github.com/DelftBioinformaticsLab/bsc-screni

- Co-clusters cells from both modalities (scRNA-seq and scATAC-seq) into one shared space.
- Finds each cell's nearest neighbours to build a cell-specific dataset.
- Links peaks to nearby genes and scans them for transcription factor binding sites.
- Trains a random forest per cell to predict each gene from other genes and peak accessibility, giving a weighted cell-specific GRN.

RESULTS

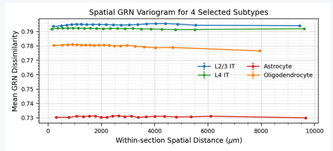


Figure 1: *Empirical variograms of GRN dissimilarity (1-cos sim) versus spatial distance band, per cell subtype. The flat curves indicate no smooth spatial gradient in whole-GRN structure.*

Subtype	Med. (µm)	≤500 µm	≤1000 µm
L2/3 IT	1435	28.9%	40.3%
L4 IT	1585	27.3%	38.7%
Oligodendrocyte	1787	24.1%	33.9%
Astrocyte	1947	22.5%	31.0%

Table 1: *Spatial mapping accuracy for the operating configuration (donor-matched, section-aware, argmax, native 140-gene panel). Median localisation error and the fraction of held-out cells placed within 500 µm and 1000 µm of their true position, per subtype.*

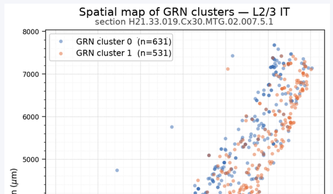


Figure 2: *Spatial map of GRN-defined clusters for L2/3 IT cells in section H21.33.019.Cx30.MTG.02.007.5.1. Each point is a cell at its inferred MERFISH coordinate, coloured by its GRN-based k-means cluster (k = 2), where clusters are assigned from the GRN principal components independently of spatial location.*

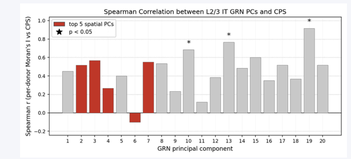


Figure 4: *Per-donor Spearman correlation between each L2/3 IT GRN principal component's Moran's I and donor CPS, across the ten donors. Red bars are the five most spatially autocorrelated components (PCs 2, 5, 4, 6 and 7), selected independently of CPS; the strongest, PC5 (ρ = 0.57), does not survive Benjamini- Hochberg correction. Asterisks mark components reaching raw p < 0.05, none of which belong to the pre-selected spatial set.*

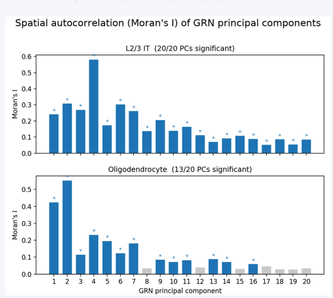


Figure 3: *Spatial autocorrelation (Moran's I) of each GRN principal component for the L2/3 IT and Oligodendrocyte subtypes. Blue bars are significantly spatially autocorrelated (p < 0.05, 999 permutations) and marked with an asterisk, while grey bars are not, and each panel heading reports the number of significant components. Positive values indicate spatial clustering of GRN states.*

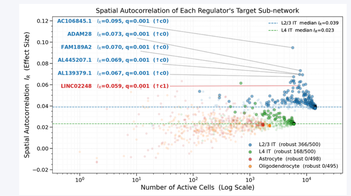


Figure 5: *Spatial autocorrelation (IR) of each regulator's target sub-network versus the number of cells in which the regulator is active (log scale), coloured by subtype. Each point is one regulator; the five most autocorrelated regulators and LINC02248 are labelled with their Benjamini-Hochberg q-values, and the arrows (↑ 0/↑ 1) indicate the GRN cluster (Figure 2) in which the regulator is stronger. Dashed lines mark the per-subtype median IR.*

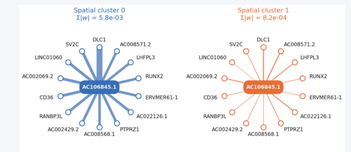


Figure 6: *Mean target sub-network of regulator AC106845.1 across the two GRN clusters (Figure 2) in L2/5 IT, drawn on a shared edge-width scale. The same target genes are present in both clusters and the network shape is preserved, while the overall edge magnitude, proportional to edge width, differs markedly between clusters (mean |w| = 0.0058 versus 0.0008)*

DISCUSSION

- GRN structure is non-randomly organised in tissue space. Spatially close cells share more similar GRN states than chance. This is not a smooth gradient but discrete, spatially contiguous clusters.
- The signal is carried by specific regulators concentrated in the neuronal subtypes, and absent in glia. Oligodendrocytes are vulnerable in AD yet show no spatial regulator structure, so spatial patterning does not simply track cell-type vulnerability.
- The mechanism is on/off, not rewiring. Target shape is preserved (cosine 0.8 to 0.9) while total magnitude changes. AC106845.1 is switched on in one cluster and off in the other. The effect is regulator-specific (3 to 7x over a 1.3x background), not a global gradient.
- LINC02248 runs against the crowd, switched on in the opposite cluster to the bulk of regulators, which also rules out a global gradient explanation.

CONCLUSION

- GRN structure is spatially organised in AD tissue, driven by specific regulators in the neuronal subtypes. It could not be reliably linked to disease severity at this scale due to lack of donor-level observations.
- This shows single-cell GRNs can be inferred and spatially analysed by combining ScReNI and Tangram, at a finer resolution than the cell-type and cell-cluster methods available before.
- Spatial mapping held up as a proof of concept, placing 22 to 29% of held-out cells within 500 µm of their true location. Noting that the lowest error placements contain the most spatial gene expression information.

LIMITATIONS & FUTURE WORK

- Both the positions and the networks are inferred, not measured, and localisation is coarse (median 1.4 to 1.9 mm). Further research and validation is needed.
- Ten donors, a 9 to 1 male skew, no neurotypical controls, and MTG tissue only. This leaves donor-level severity claims underpowered and limits how far the findings generalise.
- Future work: extend to more subtypes and datasets, add AD controls and a sex-balanced cohort, and investigate the top regulators (AC106845.1, ADAM28, FAM189A2, LINC02248).
- A larger donor cohort would be needed to test the GRN-severity link properly.