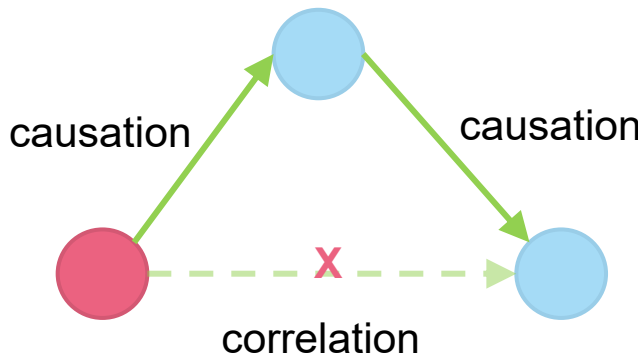


Danni Liu, Zhongli Jiang, Hyunjin Kim, Anke M. Tukker, Ashish Dalvi, Junkai Xie, Yan Li, Chongli Yuan, Aaron B. Bowman, Dabao Zhang, Min Zhang
Department of Epidemiology and Biostatistics, University of California, Irvine

Background

- Alzheimer's disease (AD) involves complex regulatory disruptions across multiple brain cell types, yet the comprehensive intracellular causal mechanisms remain poorly understood.
- Prior studies for constructing gene regulatory networks (GRNs) have relied on correlation-based models with transcriptional factor (TF)-centric assumptions, which often yield a limited network mapping with a high rate of false positive associations rather than causal regulations.



Cohort & Methods

Cohort: 272 AD patients from the Religious Orders Study and Rush Memory and Aging Project (ROSMAP)¹, who have both snRNA-seq and whole genome sequencing (WGS) data from dorsolateral prefrontal cortex (DLPFC) tissue. (*Table 1*)

Methods: We employed the SIGNET^{2,3} computational model, which leverages the power of genomic variants as cell-type-specific instrumental variables (ctIV) to establish robust causal relationships between genes. (*Figure 1*)

- Identified ctIVs through cis-eQTL mapping, stratified by minor allele frequency (MAF) to ensure reliable analysis of rare alleles;
- Constructed cell-type-specific causal GRNs using a transcriptome-wide Mendelian Randomization model.³

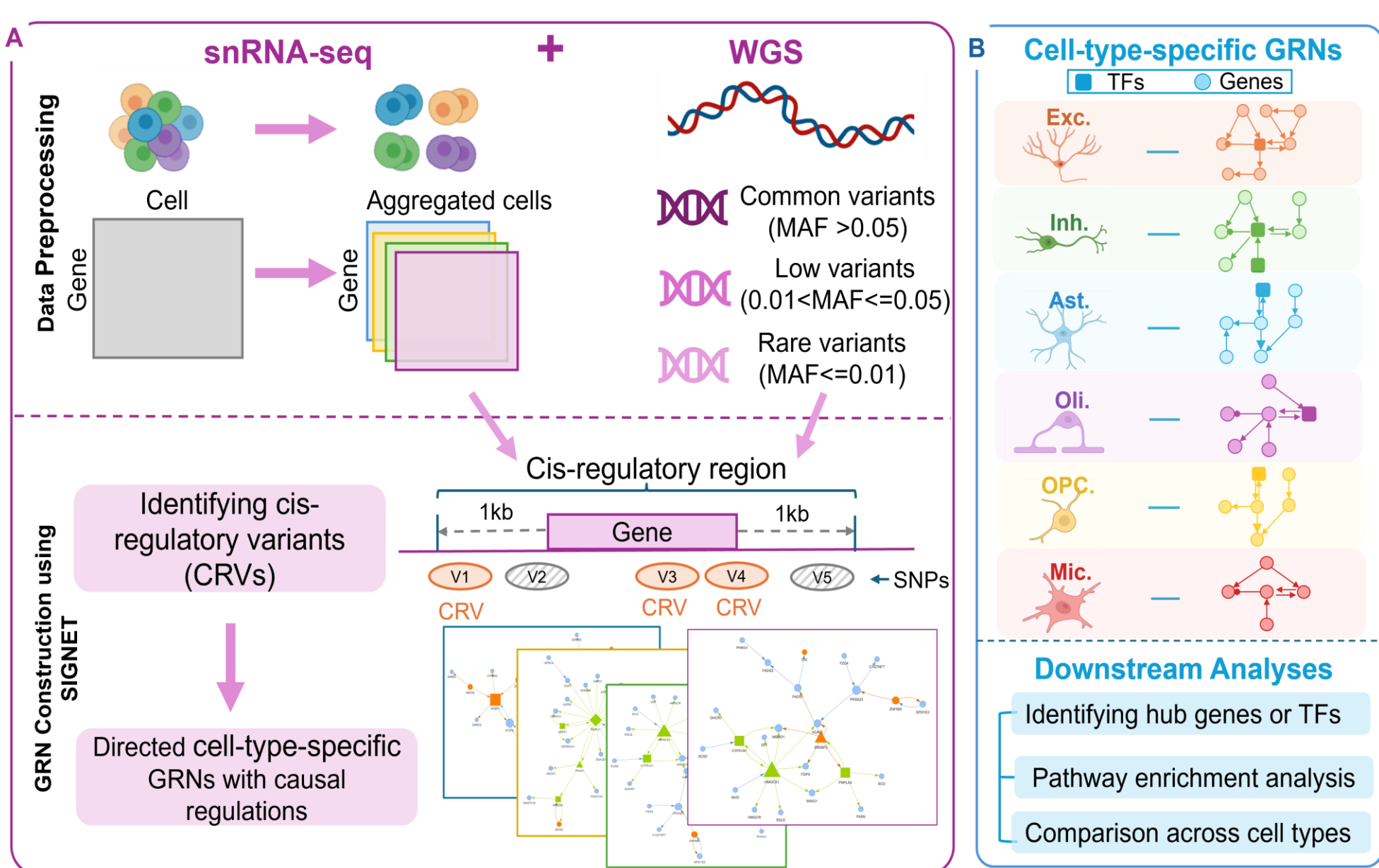
Table 1. Demographic Characteristics of the ROSMAP cohort
Categorical Demographic variables

Variable	Category	N (%)
Study	ROS (%)	135 (49.63%)
	MAP (%)	137 (50.37%)
Gender	Male (%)	77 (28.31%)
	Female (%)	195 (71.69%)
APOE genotype	$\epsilon 4/\epsilon 4$ (%)	4 (1.47%)
	$\epsilon 4/\epsilon 3$ (%)	80 (29.41%)
	$\epsilon 4/\epsilon 2$ (%)	6 (2.21%)
	$\epsilon 3/\epsilon 3$ (%)	148 (54.41%)
	$\epsilon 3/\epsilon 2$ (%)	34 (12.50%)
Age	≥ 90	141 (51.84%)
	< 90	131 (48.16%)

Numeric demographic variables	
Variable	Mean $\pm$ SD (Min, Max)
Age* (Under 90)	85.11 $\pm$ 3.93 (71.31, 89.96)
MMSE** score	19.49 $\pm$ 9.12 (0, 30)
PMI*** (Hours)	7.90 $\pm$ 5.42 (1.50, 40.80)

* Individuals aged 90 and above were recorded as 90+.
**MMSE: Mini-Mental State Examination with 11 cognitive screening questions for assessing cognitive impairment levels.
***Postmortem time interval (PMI) refers to the period between death and tissue preservation, measured in hours.

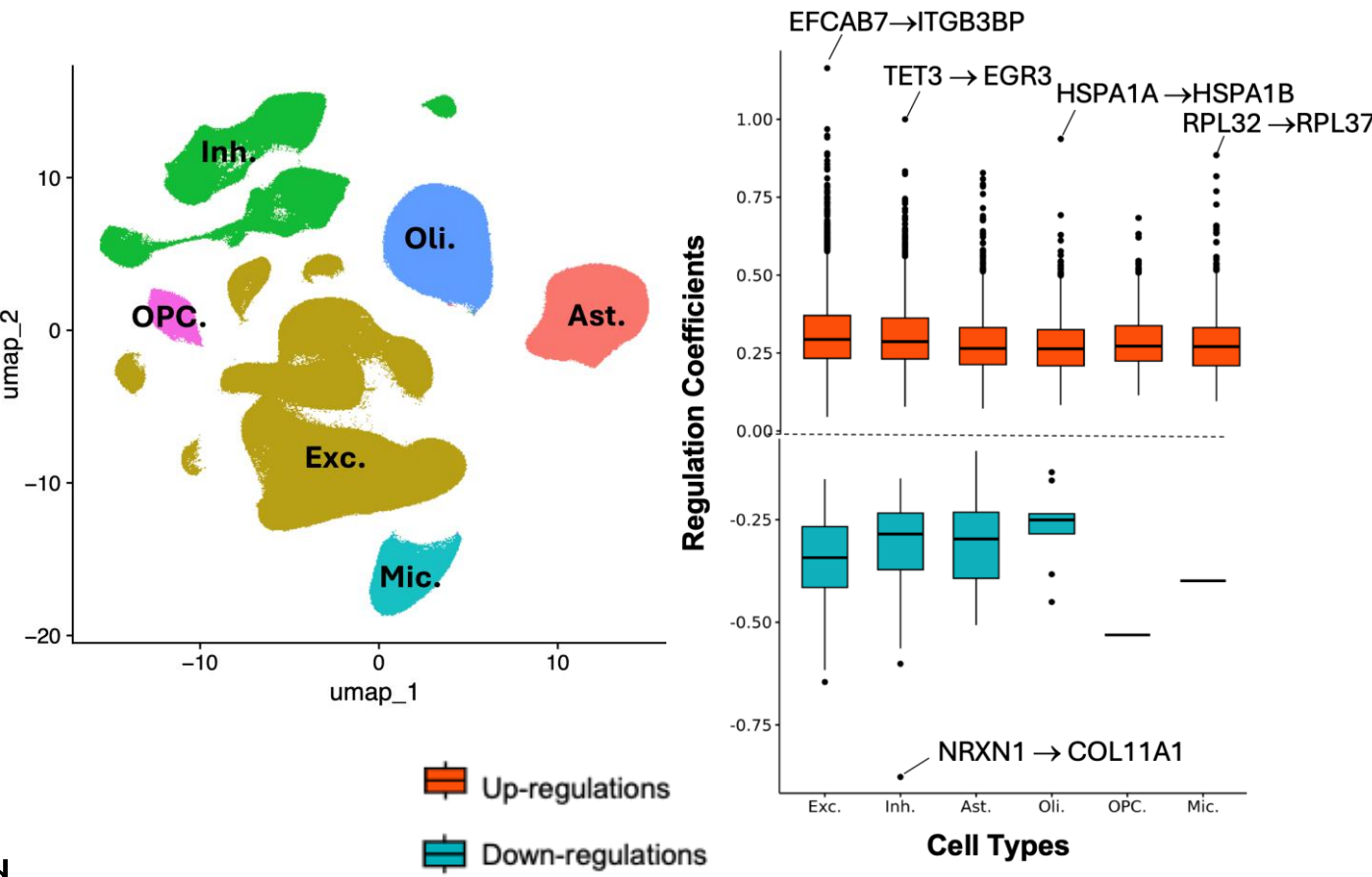
Figure 1. Analysis pipeline of cell-type-specific causal GRN construction.



Results

- Our study constructed causality-based, comprehensive cell-type-specific GRNs of AD across six major brain cell types, including excitatory neurons (Exc.), inhibitory neurons (Inh.), astrocytes (Ast.), microglia (Mic.), oligodendrocytes (Oli.), and oligodendrocyte progenitor cells (OPC.) Excitatory neurons present the largest GRNs with the strongest regulatory effects in both directions. (*Figure 2*)

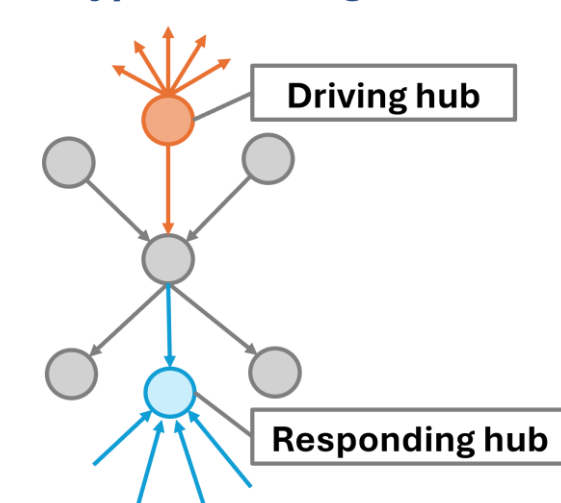
Figure 2. A UMAP of cell types and GRN coefficients boxplots across cell types



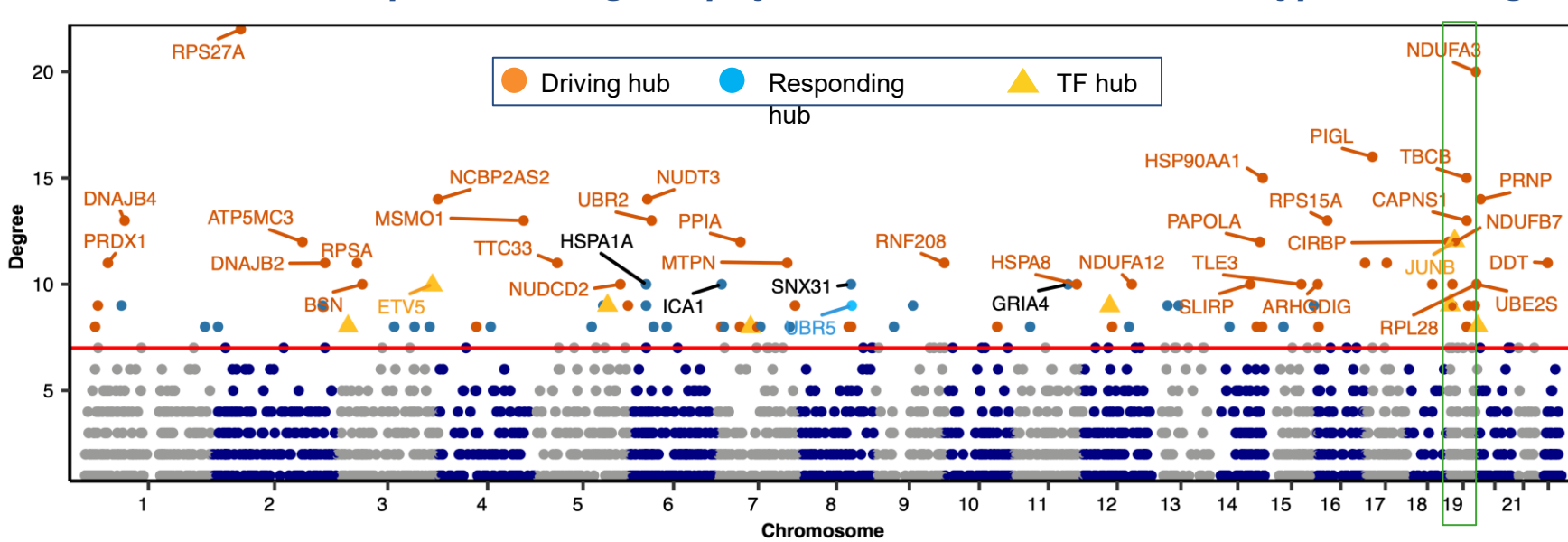
- Hub gene roles as regulators or targets were quantitatively evaluated within cell-type-specific GRNs.⁴ (*Figure 3*)
 - Top hub genes in excitatory neuron GRN include driving hubs *RPS27A*, *NDUFA3* and TF hubs *JUNB* and *ETV5*.
 - A notable genomic hotspot was observed in chromosome 19 with multiple driving hubs and TF hubs.

Figure 3. Hub genes in excitatory-neuron-specific GRNs

A. Types of hub genes



B. A Manhattan plot showing the physical locations of different types of hub genes

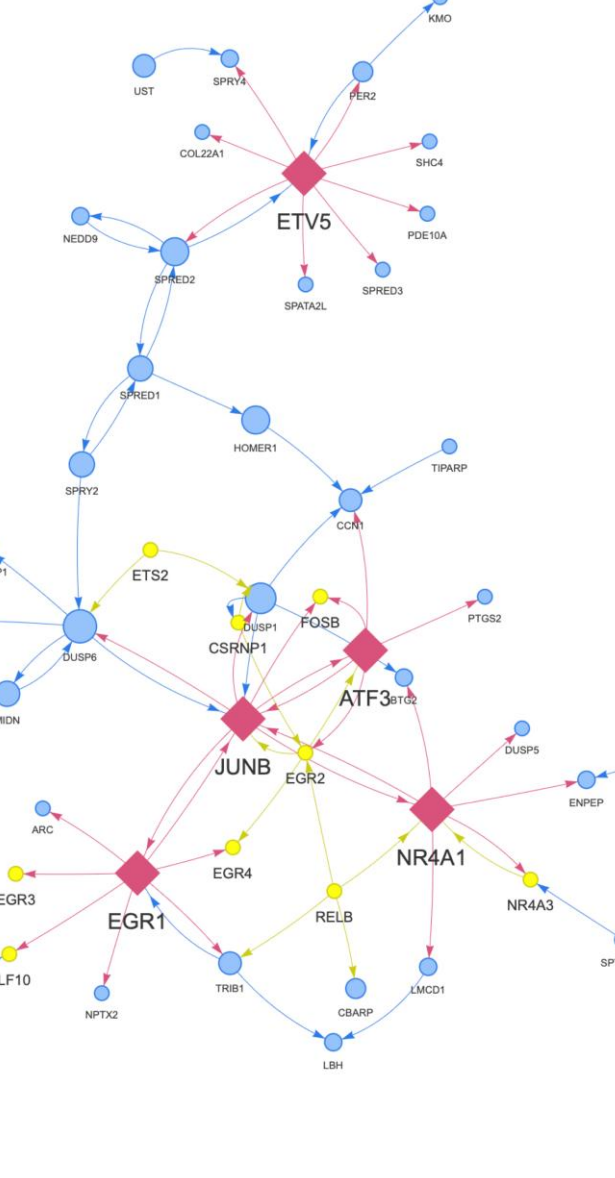


- A tightly interconnected regulatory circuit was observed among five TF hubs (*JUNB*, *ETV5*, *EGR1*, *ATF3*, and *NR4A1*) in excitatory neurons, which interact with many secondary TFs and simultaneously coordinate the rest of the genes (*Figure 4A*)

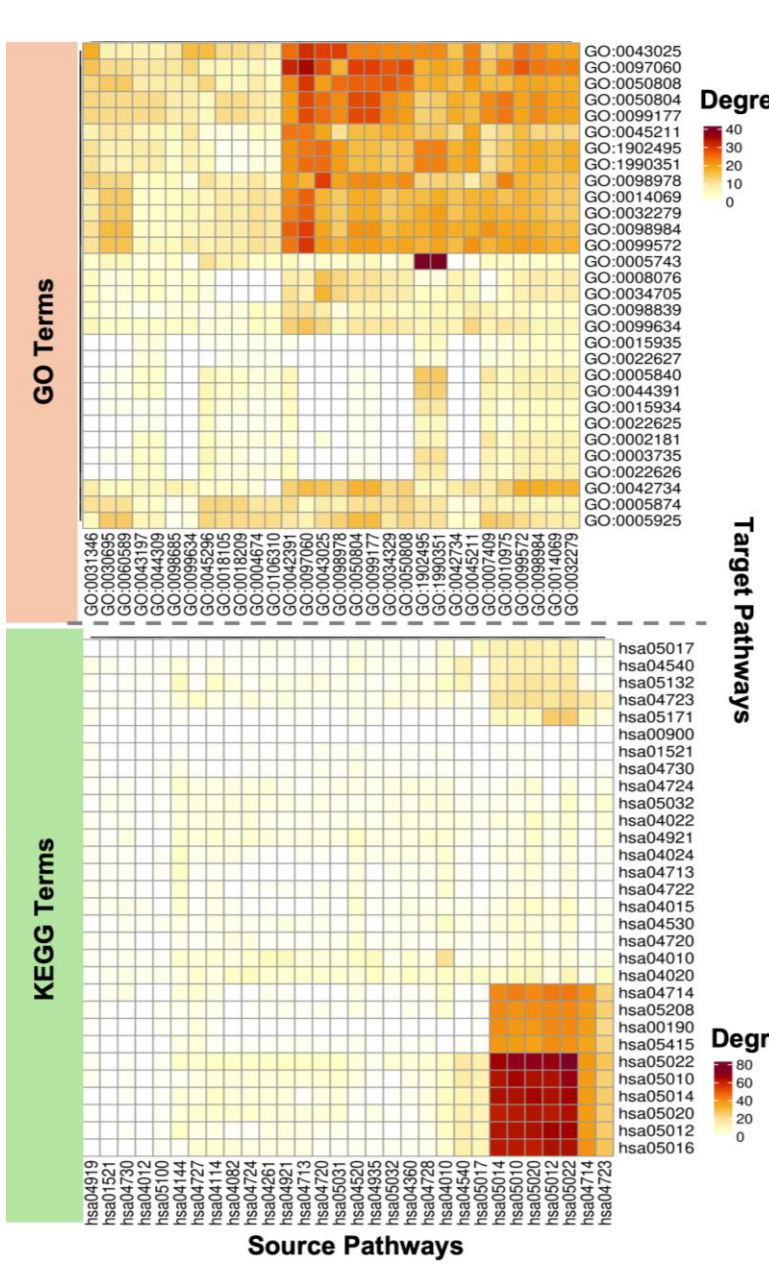
- Pathway analysis of the causal GRNs show directed pathway regulatory networks. (*Figure 4B*)
 - GO analysis shows prominent regulations from the transporter complex to the mitochondrial inner membrane, and dense interconnections among pathways related to synaptic organization, neuronal cell body organization, and glutamatergic synaptic transmission.
 - KEGG analysis reveals enrichment in neurodegenerative disease pathways, particularly Alzheimer's and Parkinson's disease.

Figure 4. A zoom-in GRN of TFs and pathway analysis of excitatory neurons

A. GRN of selected TF hubs (*JUNB*, *ETV5*, *EGR1*, *ATF3*, and *NR4A1*) and their two nearest neighbors.



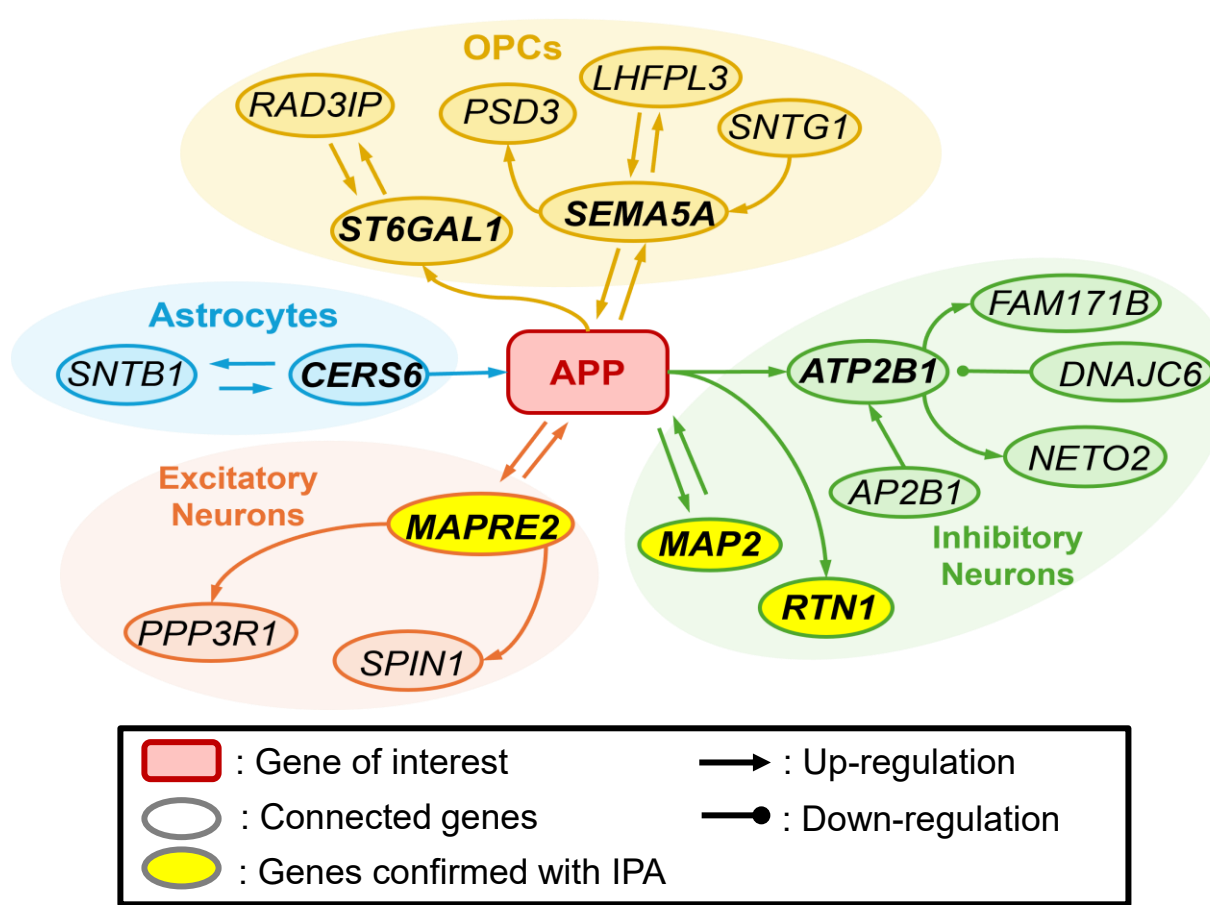
B. Pathway enrichment signals from upstream regulators to their targets.



Results (continued)

- Gene *APP* acts as a strong causal regulator in inhibitory neurons, explaining 73.01% variation in gene *RTN1* and 43.30% in *MAP2*.
- Our GRN completes and refines existing protein-protein interaction networks in IPA, providing causal directionality for experimentally supported interactions.

Figure 5. APP-involved regulations across cell types, with genes confirmed to have connections in QIAGEN IPA highlighted in yellow.



Discussion

- We identified upstream non-TF regulators controlling TF expression and interconnected TF regulatory modules, demonstrating the complexity of AD regulatory mechanisms and the necessity of moving beyond classical TF-centric assumptions.
- Our study provides a comprehensive map of cell-type-specific causal GRNs in AD, with a methodology applicable to other complex diseases such as cancer, enabling dynamic pathway exploration, hypothesis generation, and functional interpretation.

Funding & Acknowledgements

Funding: NIH grants R01AG080917, R01AG080917-02S1, R01GM131491, and R01GM131491-02S2, NIEHS grant K99ES036290, and P30CA062203.

Acknowledgements: The results published here are in whole or in part based on data obtained from the AD Knowledge Portal Community Data Contribution Program.

References

- Bennett DA, Buchman AS, Boyle PA, Barnes LL, Wilson RS, Schneider JA. Religious Orders Study and Rush Memory and Aging Project. J Alzheimers Dis. 2018;64(s1):S161-S189.
- Jiang Z, Chen C, Xu Z, Wang X, Zhang M, Zhang D. SIGNET: transcriptome-wide causal inference for gene regulatory networks. Sci Rep. 2023;13(1):19371.
- Chen C, Ren M, Zhang M, Zhang D. A two-stage penalized least squares method for constructing large systems of structural equations. J Mach Learn Res. 2018;19(2):1-34.
- Jiang Z, Zhang D. Analysis of variance of multiple causal networks. Advances in Neural Information Processing Systems. Vol 36. Curran Associates, Inc.; 2023:11580-11591.