

Functional organization of the transcriptome in human brain

Oldham MC, Konopka G, Iwamoto K, Langfelder P, Kato T, Horvath S, Geschwind DH. Nature Neurosci. 2008 Nov;11(11):1271-82. Epub 2008 Oct 12.

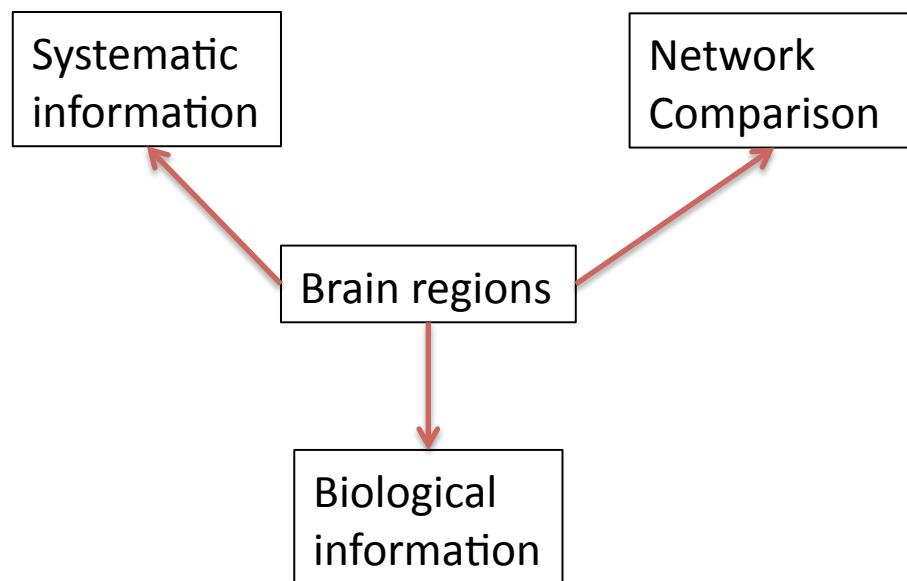
DW

Journal Club Feb 21st 2012

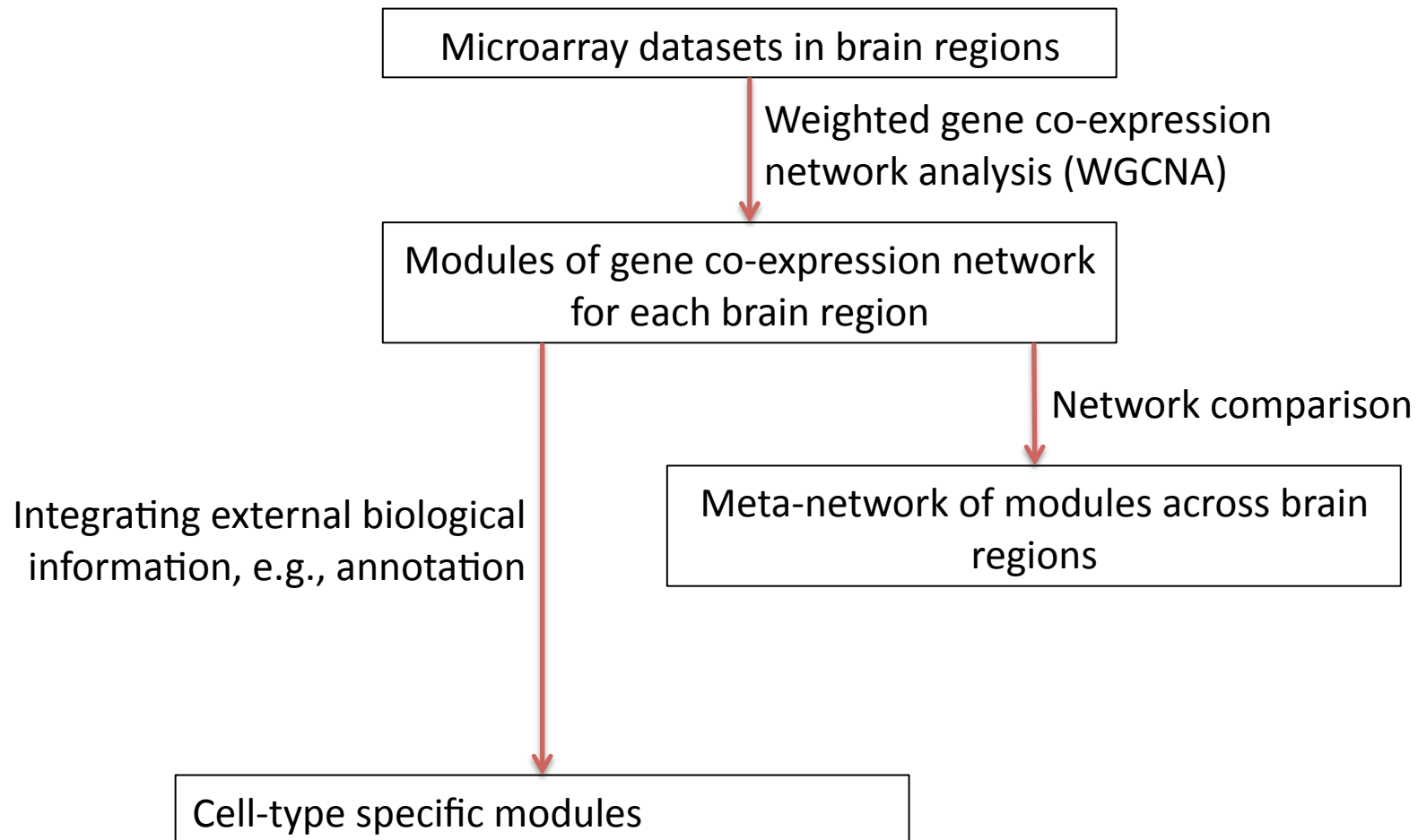
Gerstein lab

Motivation

- Integrated view of transcriptome organization across human brain regions



Methods



Microarray Datasets

Brain Region	Number of genes	Number of samples	Microarrays
A) CTX: Cortex Brodmann area	5549	67	Affymetrix U133A
B) CTX_95: Different cortex Brodmann area	3202	42	Affymetrix U95A
C) CN: Caudate nucleus (head)	4050	27	Affymetrix U133A
D) CB: Cerebellum (hemisphere)	4029	24	Affymetrix U133A

Weighted Gene Co-Expression Network Analysis

- WGCNA from Dr. Horvath lab in UCLA
 - <http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/>
 - Cluster genes using gene expression variability across samples
 - Systematic analysis, R package available
- Construct co-expression network for each region
 - **Scale-free topology** and **Node dissimilarity**
 - Adjacency matrix $A=[a_{i,j}]$, where the similarity measure function $a_{i,j}$ between gene i and j : $a_{i,j} = |(1+\text{corr}(i,j))/2|^\beta$ for example
 - Node dissimilarity, Topological overlap matrix (TOM):

$$TOM_{ij} = \frac{\sum_u a_{iu} a_{uj} + a_{ij}}{\min(k_i, k_j) + 1 - a_{ij}}$$

$$DistTOM_{ij} = 1 - TOM_{ij}$$

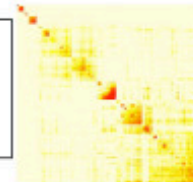
$$k_i = \sum_{j=1}^n a_{ij}$$

Overview of WGCNA

Construct a gene co-expression network

Rationale: make use of interaction patterns among genes

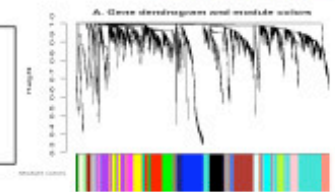
Tools: correlation as a measure of co-expression



Identify modules

Rationale: module (pathway) based analysis

Tools: hierarchical clustering, Dynamic Tree Cut

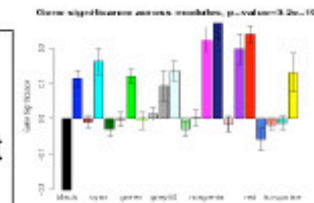


Relate modules to external information

Array Information: clinical data, SNPs, proteomics

Gene Information: ontology, functional enrichment

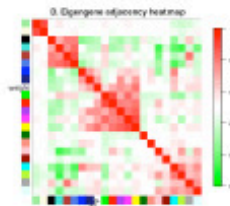
Rationale: find biologically interesting modules



Study module relationships

Rationale: biological data reduction, systems-level view

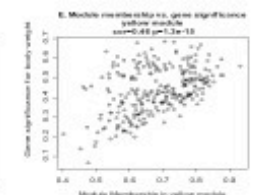
Tools: Eigengene Networks



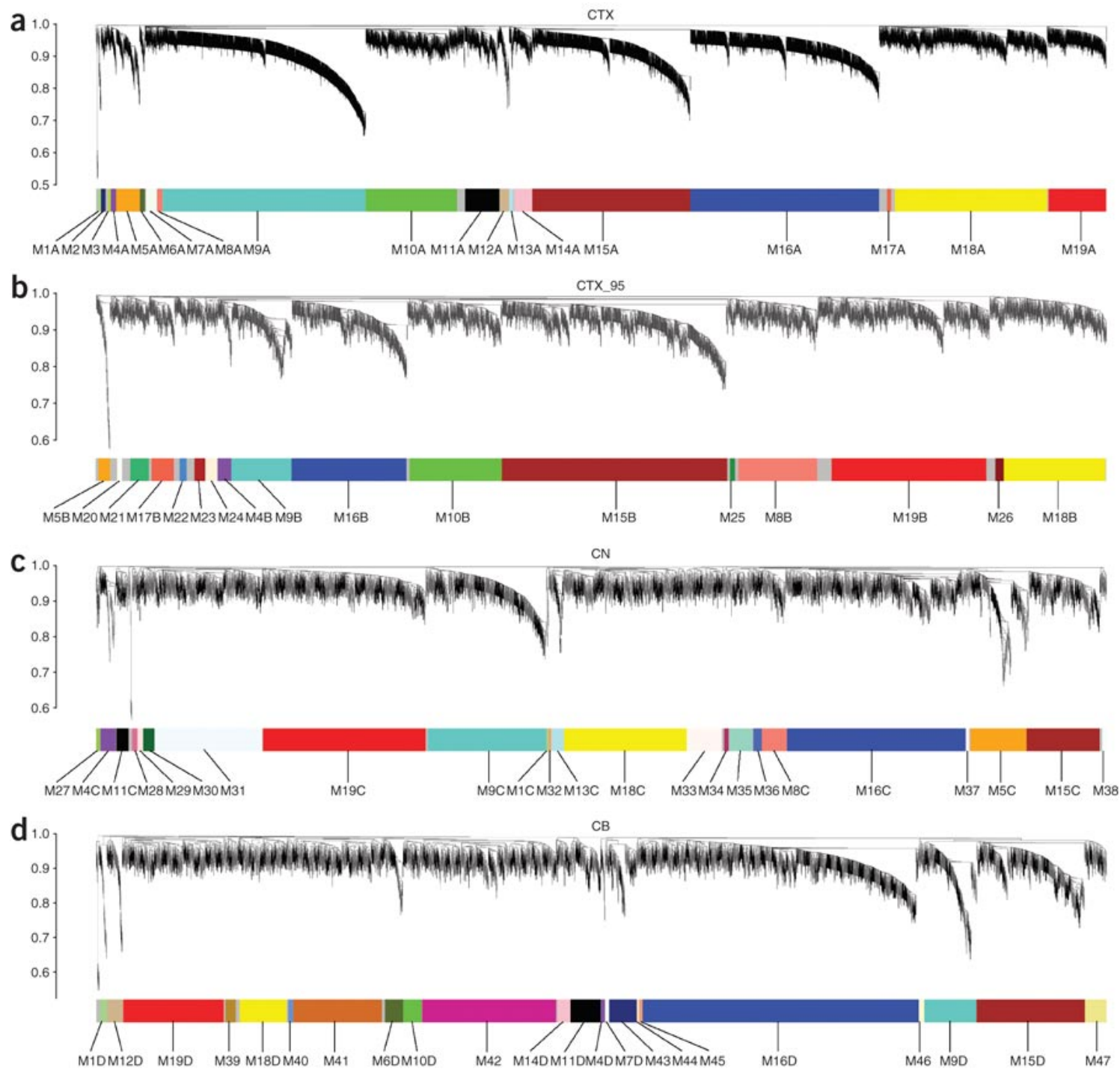
Find the key drivers in interesting modules

Rationale: experimental validation, biomarkers

Tools: intramodular connectivity, causality testing

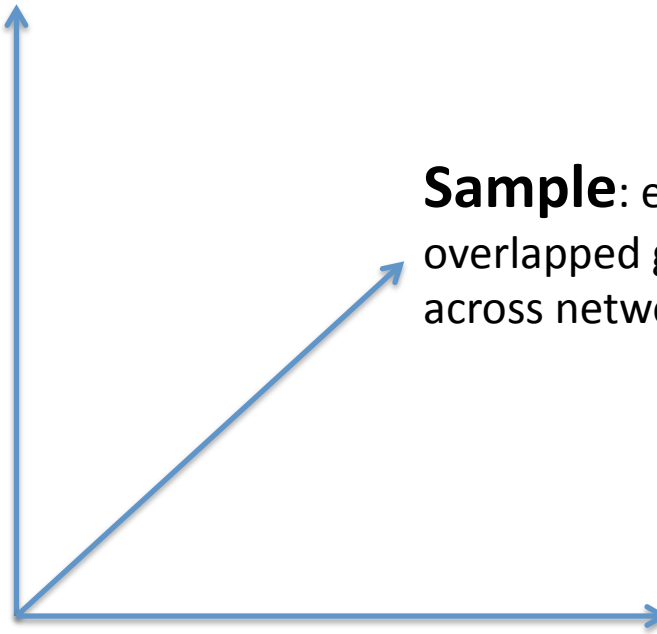


Gene co-expression modules of each network



Network comparison

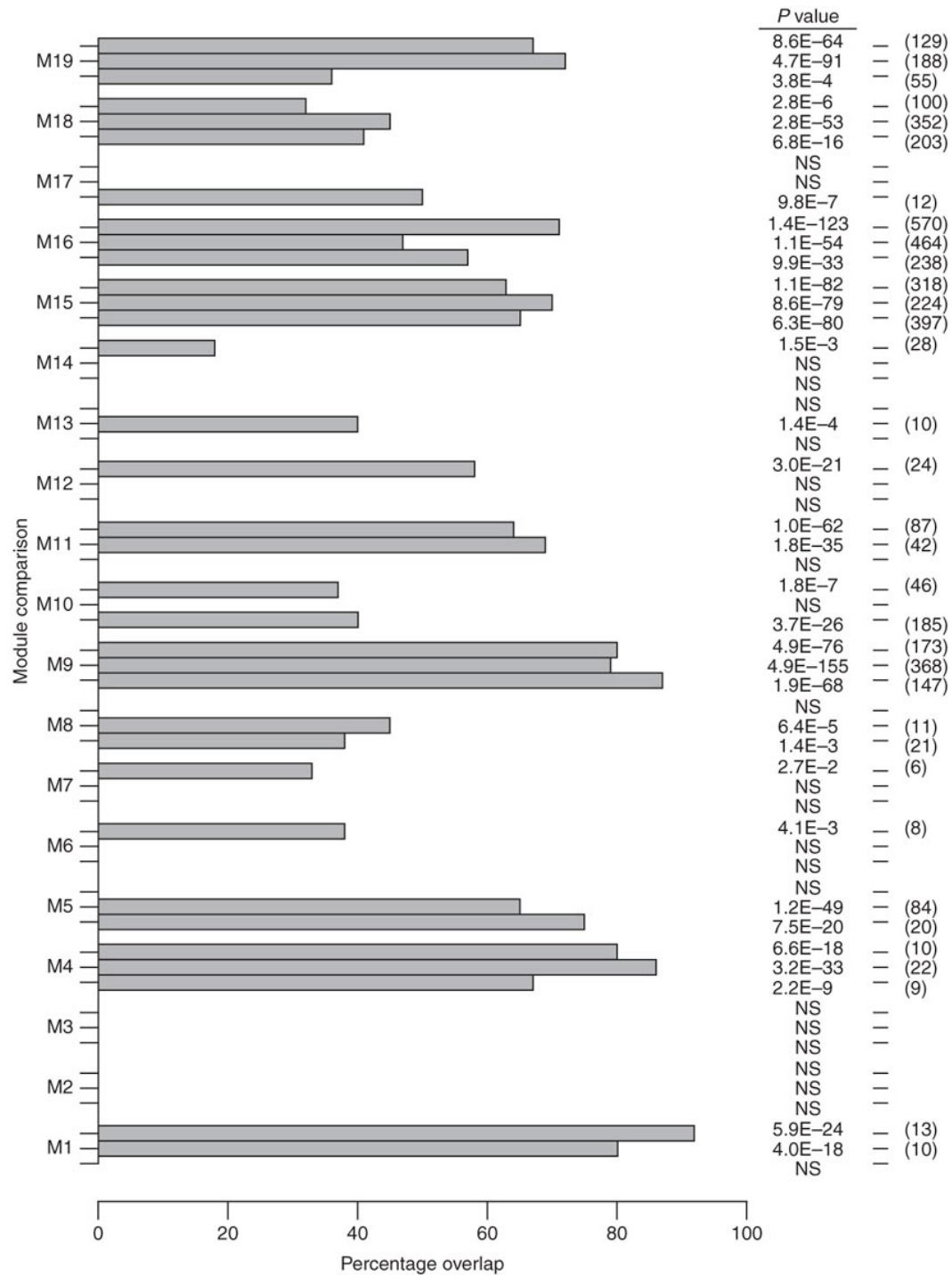
Module:
overlaps
between
modules
across
networks



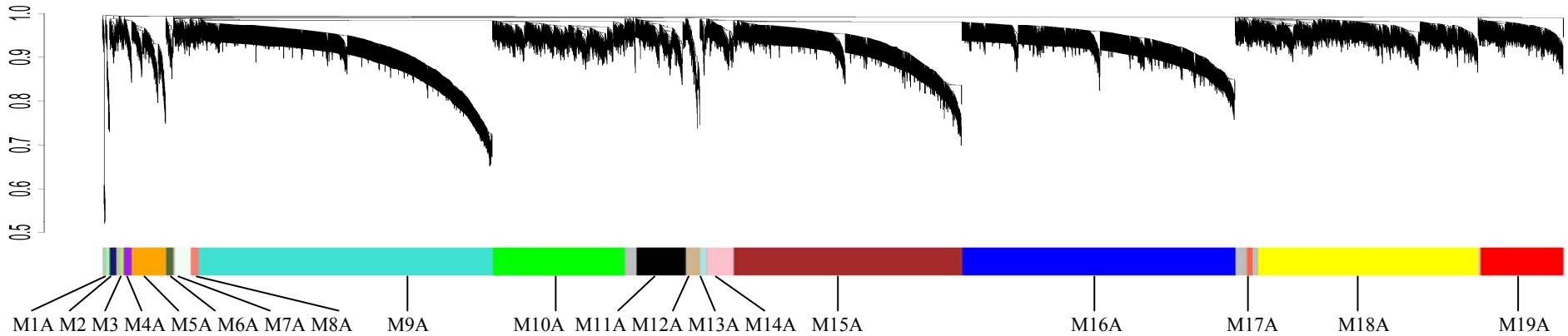
Sample: expression levels of
overlapped genes in modules
across networks

Gene: associations of genes
to modules across networks

Many modules significantly overlapped across multiple brain networks



Modules have functions

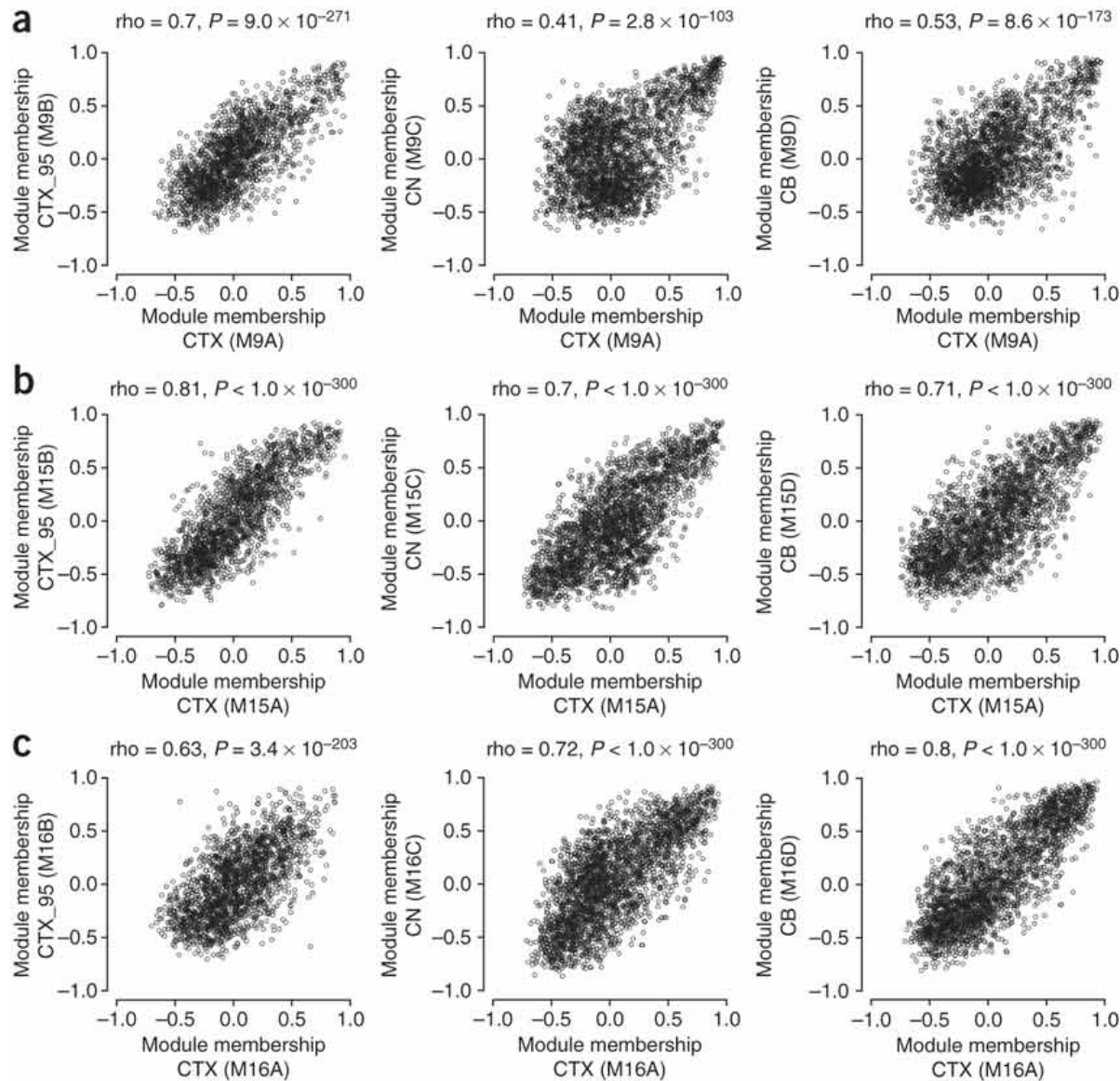


Module	Color	Interpretation	Characterization method	P-value
M1A	palegreen	Gender	Correlation with gender status	4.8e-21
M2	midnightblue	Ribosome	Gene ontology	2.3e-64
M3	greenyellow	?	N/A	N/A
M4A	purple	Immune response: microglia	Gene ontology	2.6e-56
M5A	orange	Immune response: microglia	Gene ontology	9.8e-42
M6A	darkolivegreen	PVALB+ interneuron	PVALB kme	5.3e-15
M7A	honeydew	Mitochondria	Gene ontology	2.0e-16
M8A	salmon	?	N/A	N/A
M9A	turquoise	Oligodendrocyte	Enrichment analysis with cell-type markers	8.1e-71
M10A	green	Glutamatergic synapse	Enrichment analysis with synaptic proteins	5.3e-18
M11A	black	?	N/A	N/A
M12A	tan	Hypoxia	Enrichment analysis with hypoxia genes	5.6e-13
M13A	powderblue	Neurogenesis	Gene ontology	5.0e-03
M14A	pink	Glutamatergic synapse	Enrichment analysis with synaptic proteins	1.6e-20
M15A	brown	Astrocyte	Enrichment analysis with cell-type markers	2.0e-122
M16A	blue	Neuron	Enrichment analysis with cell-type markers	8.4e-30
M17A	tomato	PVALB+ interneuron	PVALB kme	2.0e-06
M18A	yellow	Metabolism	Gene ontology	2.1e-07
M19A	red	Membrane/signal transduction	Gene ontology	6.8e-08

Gene expression modular associations highly consistent across regions

- Module membership
 - Describe how gene expression conforms to the module pattern for individual gene, i.e., the extent to which a gene “belongs” to a module
 - Correlation with 1st eigengene, i.e., the first principal component via SVD in the module
 - Conformation of gene to the systematic pattern, covarying direction across samples in its module

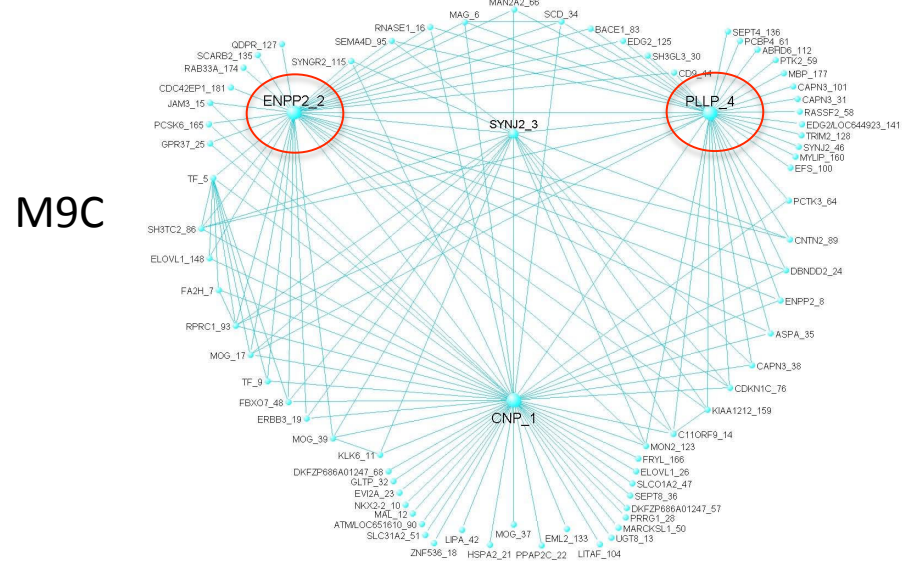
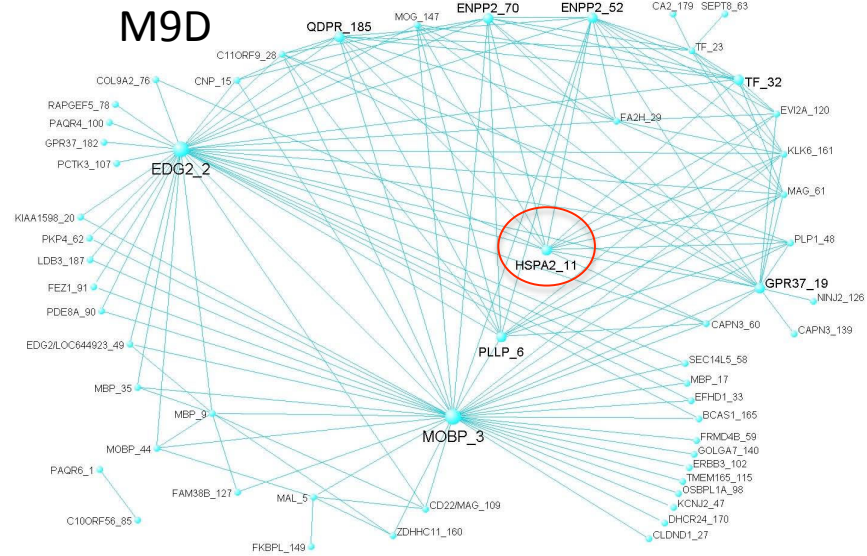
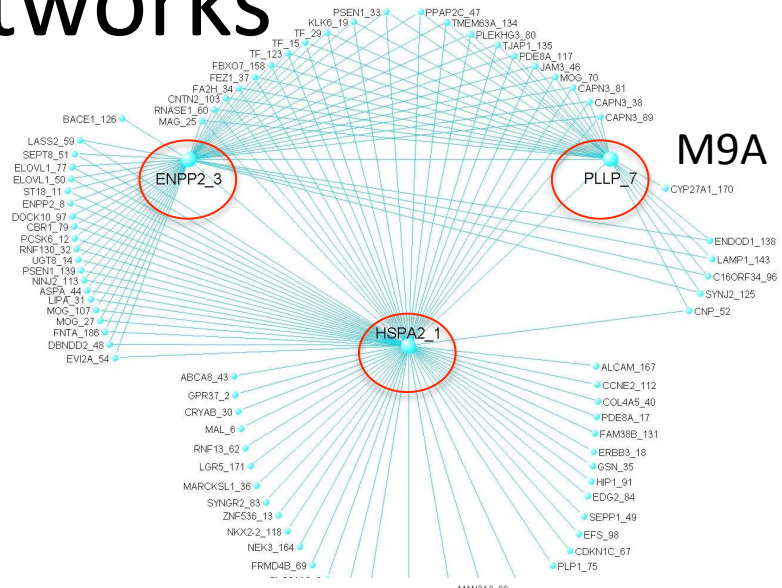
Module membership highly consistent



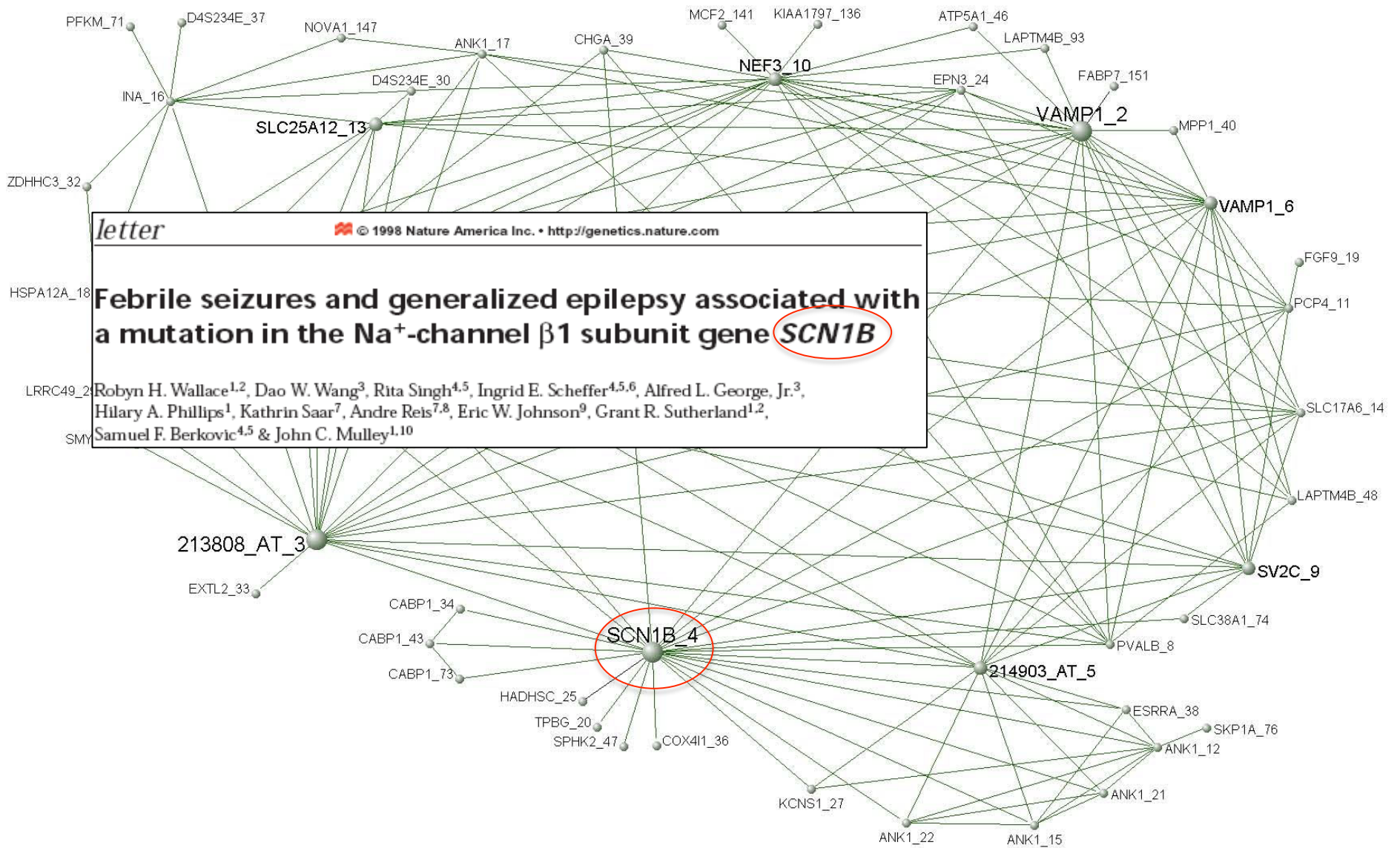
Gene's network position (role) is highly reproducible across brain regions

Hub genes in conserved modules across networks

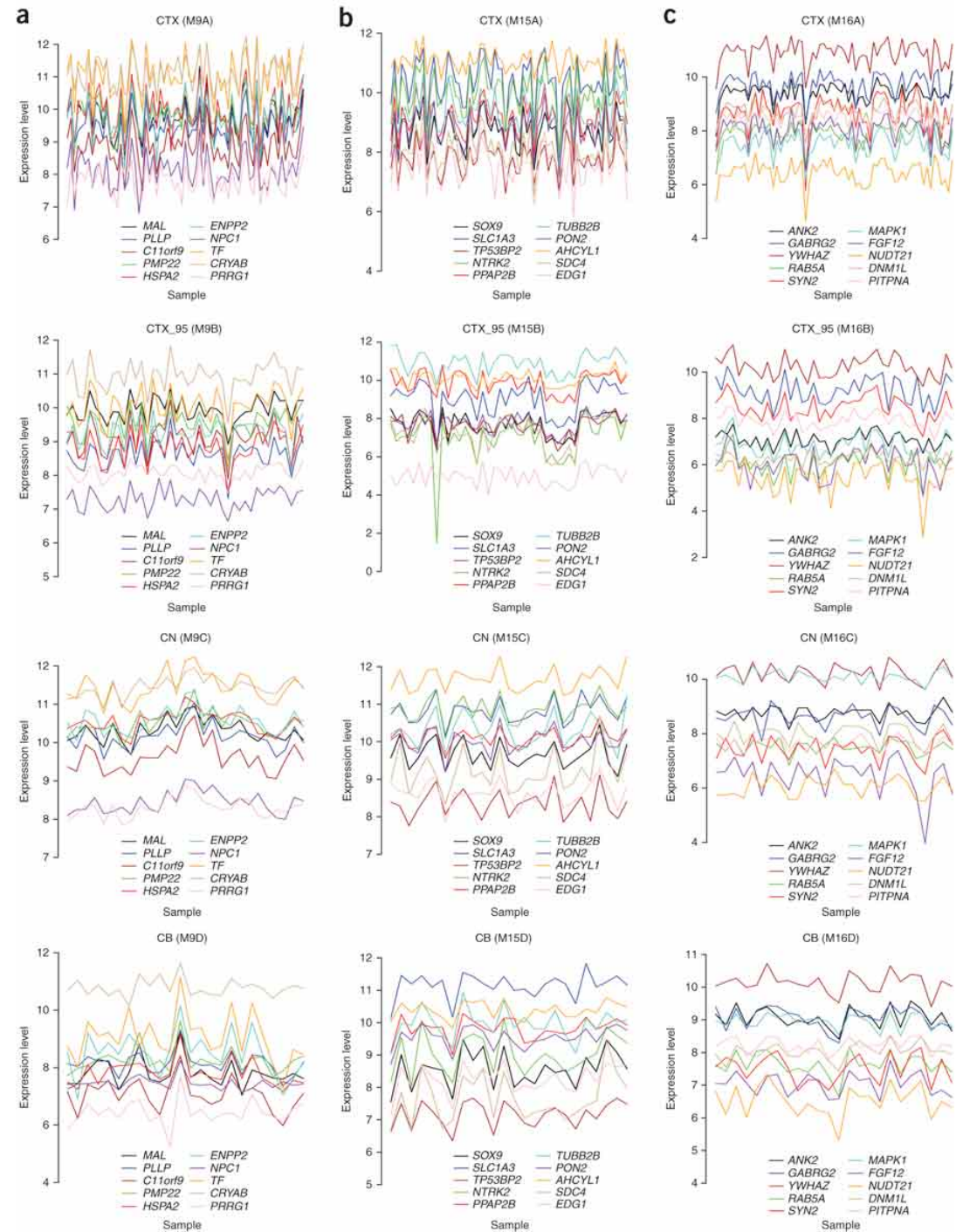
- “Guilt by association”
 - Genes with high memberships in conserved modules are driven by the same factor
 - Hub genes in M9 for example



Hub genes in M6A



Gene expression levels of genes with high module memberships across networks



Cell-type enrichment analysis

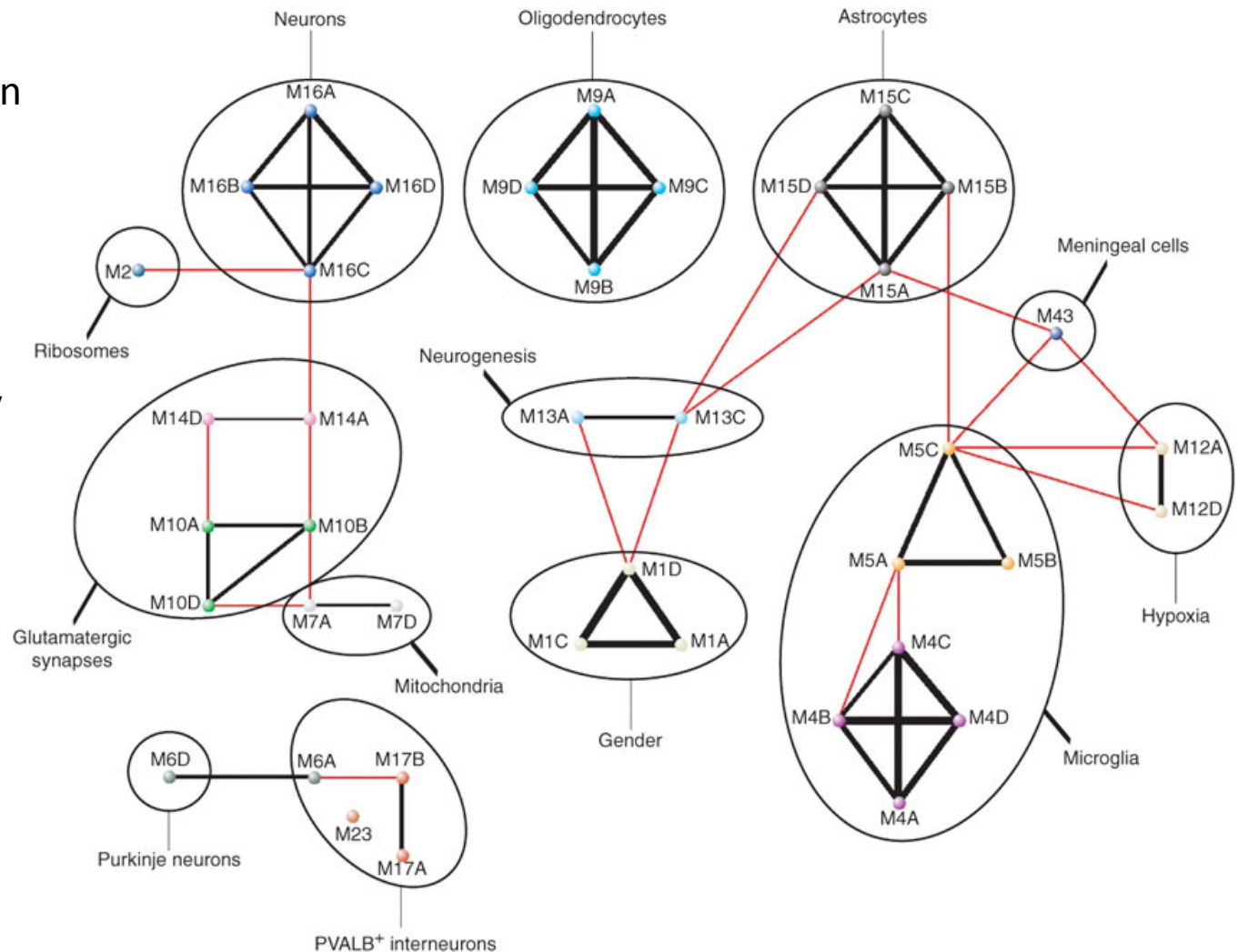
- Genes with high module memberships are enriched by certain cell types

Network	Module	Color	Oligodendrocytes ¹	Oligodendrocytes ²	Astrocytes ¹	Astrocytes ²	Neurons ¹	Neurons ²
CTX	M9A	turquoise	138 / 352 p = 8.1e-71	47 / 60 p = 8.3e-43	NS	NS	NS	NS
CTX95	M9B	turquoise	63 / 256 p = 4.3e-37	29 / 55 p = 1.1e-27	NS	NS	NS	NS
CN	M9C	turquoise	93 / 352 p = 8.8e-66	29 / 60 p = 2.1e-28	NS	NS	NS	NS
CB	M9D	turquoise	58 / 352 p = 5.6e-38	30 / 60 p = 2.2e-35	NS	NS	NS	NS
CTX	M15A	brown	NS	NS	220 / 554 p = 2.0e-122	19 / 32 p = 5.7e-14	NS	NS
CTX95	M15B	brown	NS	11 / 55 p = 3.2e-03	145 / 430 p = 5.3e-79	13 / 27 p = 7.2e-09	NS	NS
CN	M15C	brown	NS	NS	99 / 554 p = 2.5e-61	14 / 32 p = 6.6e-14	NS	NS
CB	M15D	brown	NS	9 / 60 p = 1.0e-04	102 / 554 p = 6.1e-69	16 / 32 p = 1.1e-17	NS	NS
CTX	M16A	blue	NS	NS	NS	NS	164 / 709 p = 8.4e-30	14 / 50 p = 2.0e-03
CTX95	M16B	blue	NS	NS	NS	NS	68 / 546 p = 4.0e-15	7 / 40 p = 2.4e-02
CN	M16C	blue	NS	NS	NS	NS	64 / 709 p = 1.7e-14	11 / 50 p = 4.4e-06
CB	M16D	blue	NS	NS	NS	NS	50 / 709 p = 5.4e-05	7 / 50 p = 4.1e-02

- Computational identification of cell-type specific information

Meta-network consisting of highly connected modules across networks

- Connection – correlation between eigengenes of modules
- Circle – Module cluster along with biological enrichment
- Black line – significantly conserved modules
- Red line – other connected modules



M13C identifies differentially expressed gene between the mature astrocytes and adult SVZ

- Module M13C in CN
 - Connect to modules enriched with astrocytes (meta-network) and do include astrocyte genes
 - However, include genes not expressed typically in subventricular zone (SVZ) astrocytes, e.g., **ALDH1L1**
 - Identify genes that expressed differentially between the mature astrocytes and the adult subventricular zone (SVZ) astrocytes

Experimental evidence for M13C

- **ALDH1L1** has high membership in M15 but low membership in M13C, also supported by experiments that **ALDH1L1** is expressed in mature astrocytes but not in SVZ astrocytes

