Discovery and Validation of Novel Targets underlying Cognitive Decline in AD – from Cohorts to Single Cell Profiling

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Acknowledgements

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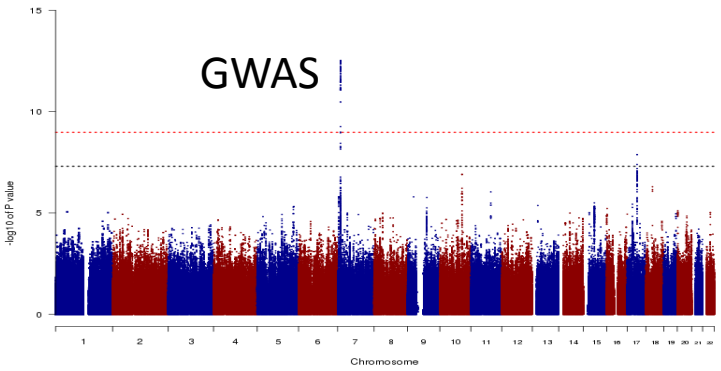
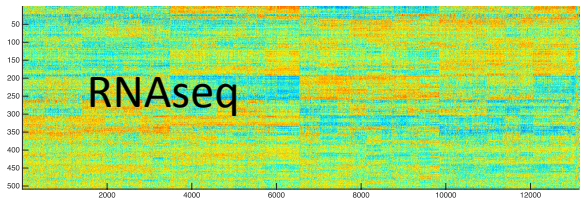
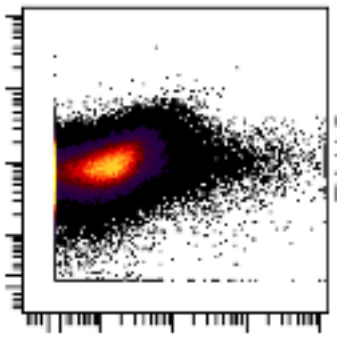
Challenge





Proteomics



Target

Neurovascular disease

Aging-related pathologies

Brain reserve & resilience

Confounders

Cohorts

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Religious Orders Study (ROS)

- Catholic priests, nuns and brothers
- Started in 1994

Memory and Aging Project (MAP)

- Older men and women in assisted living facilities in the Chicagoland area
- Started in 1997

- Free of dementia at enrollment
- Anatomical Gift Act, donate brains (spinal cords, select nerves and muscles at death)
- >3250 participants (>1350 ROS and >1900 MAP)
- >1435 autopsies
- Wide range of risk factors
- Up to 21 waves of annual cognitive function
- Annual serum, plasma, cryopreserved PBMC
- >500 with biennial imaging
- >650 cases of incident AD , >850 incident MCI
- >1200 autopsies with half brain frozen
- >750 with post-mortem imaging

PI: David Bennett

Risk Factors: Medical, Psychological, Experiential, and genome-wide genotyping

DNA methylation, histone acetylation, miRNA

mRNAseq

MS Proteomic and metabolomic

Quantitative neurobiology

Structural and function imaging

Quantitative clinical phenotype

Syndromic phenotype

Genotypes
Affy 6.0/Illumina Quad
1000G imputation

DNA Methylation
Illumina 450K
Histone H3K9Ac
ChIP-Seq

RNA profile
mIRNA & RNAseq

ATACseq
Neuronal nuclei

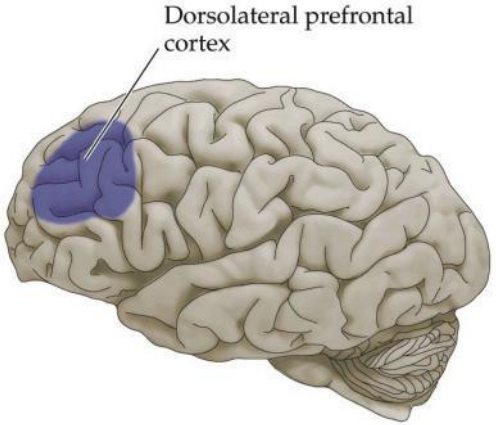
LC/MS profile
Lipids, proteins

Whole Genome Sequencing

Peripheral Monocyte RNAseq

Microglial Sequencing

Single nucleus sequencing



Neuropathology
AD, CVD, LBD, HS, TDP

Resilience Markers
Synaptic proteins, LC

Flair, MP Rage, DTI, SWI, rsfMRI

post-mortem MRI
DTI, MP Rage, T2

Clinical Diagnoses
AD, Stroke, PD,

Cognitive Function
19 tests annually

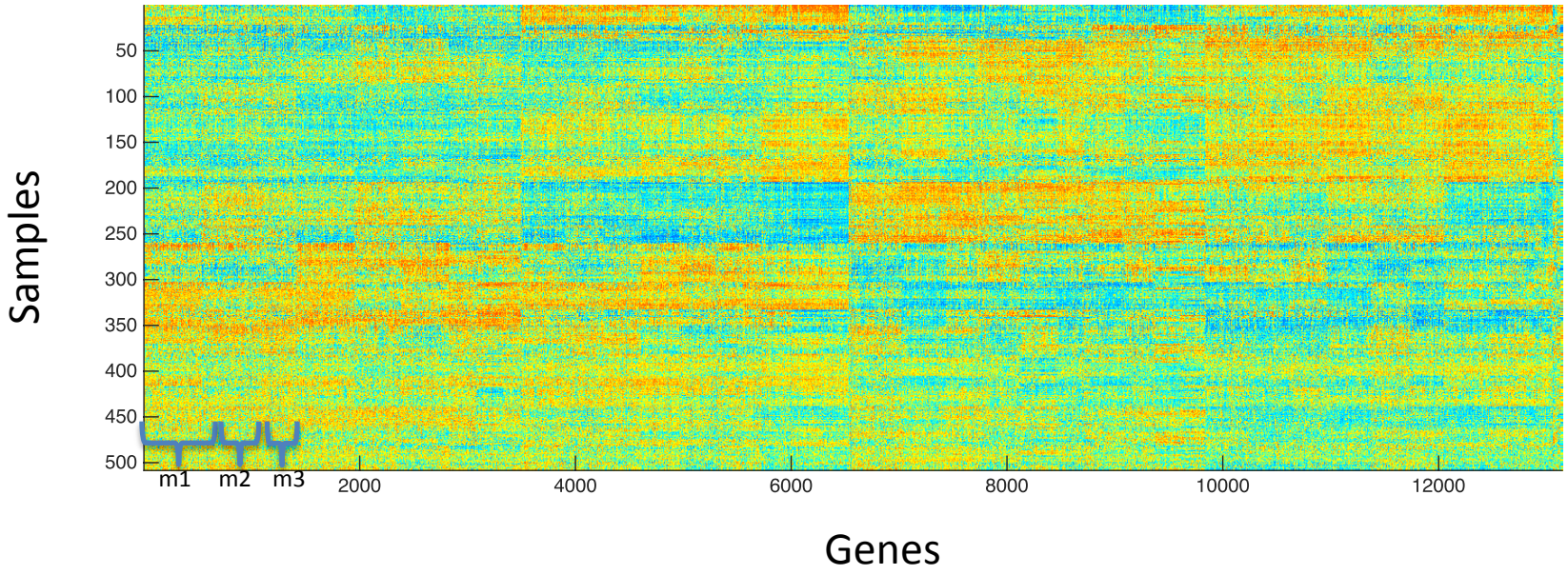
Motor Function
Disability BMI,
Actigraphy

De Jager *et al Nat Scientific Data,* in press

Establishing a network map of the aging cortex

Identifying modules of co-expressed genes

- N=540 subjects with DLPFC RNAseq data
- Identified 47 modules, ranging in size (20 to 600 gene members)
- Modules are coherent in H3K9Ac data and replicate data set



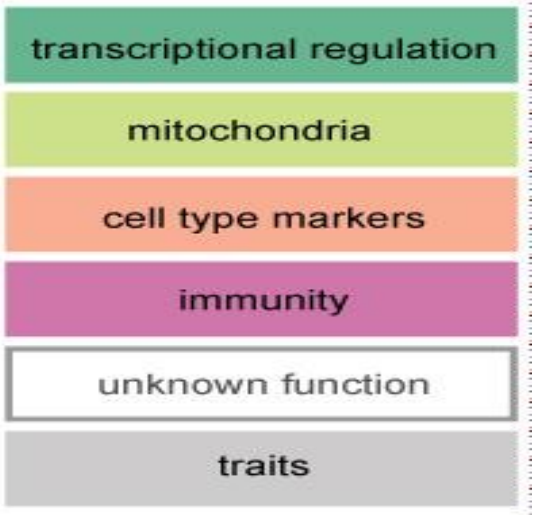
Module assignment

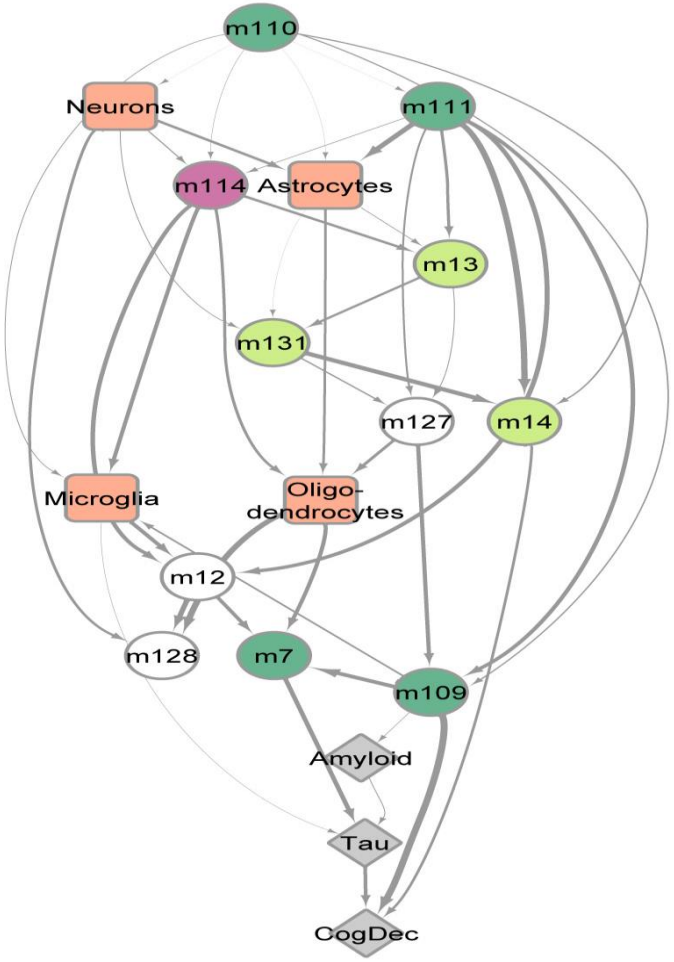
Mostafavi*, Gaiteri*, *et al Nat Neuro* 2018

Relating modules to traits

Module-Trait Network Analysis:

A directed graph describing the conditional independence relationships modules, traits, and cell composition



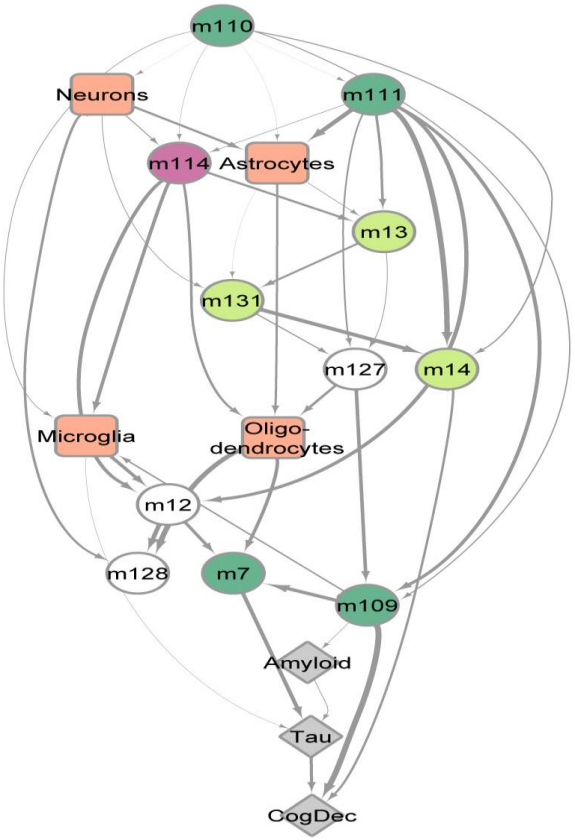


Mostafavi*, Gaiteri*, *et al Nat Neuro* 2018

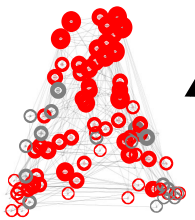
Genetic variation

ALS
Alzheimer
MS
Parkinson

Cognitive Reserve
Cognitive resilience



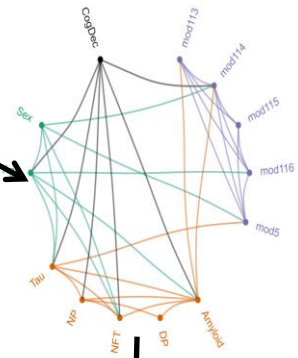
Identifying novel target genes for therapeutic development



Validated nodal genes
INPPL1
PLXNB1

Molecular network map of the aging cortex

Dissecting an immune sub-network

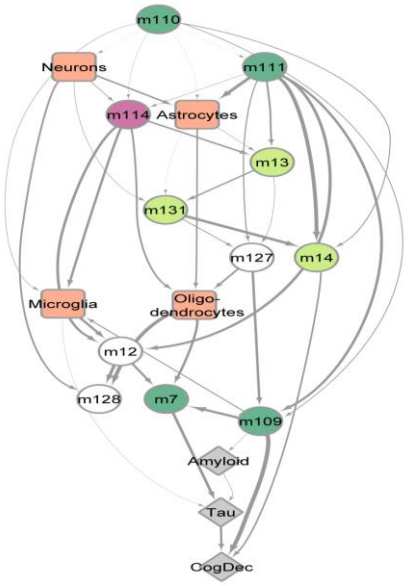


Distinct immune responses involved in amyloid & Tau

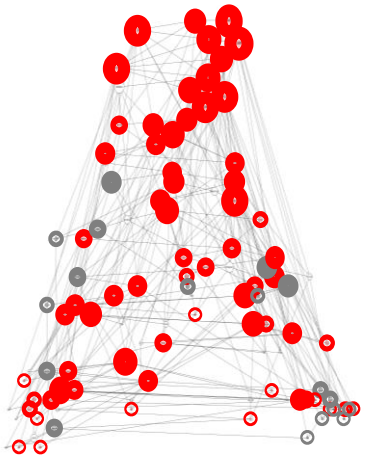
Target validation

In vitro module evaluation pipeline

Frontal Cortex
RNAseq-derived network

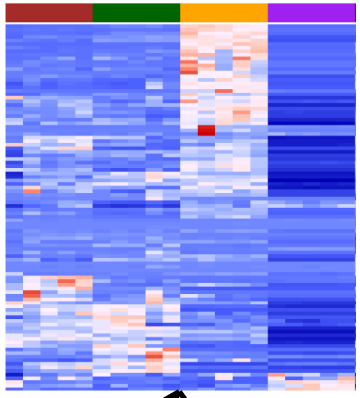


Module 109

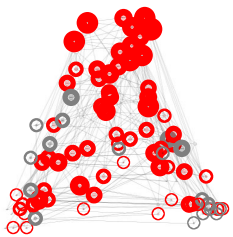


A.

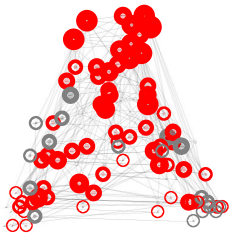
Reference model system
RNAseq profiles



iPSC-derived neurons



Astrocyte cell line



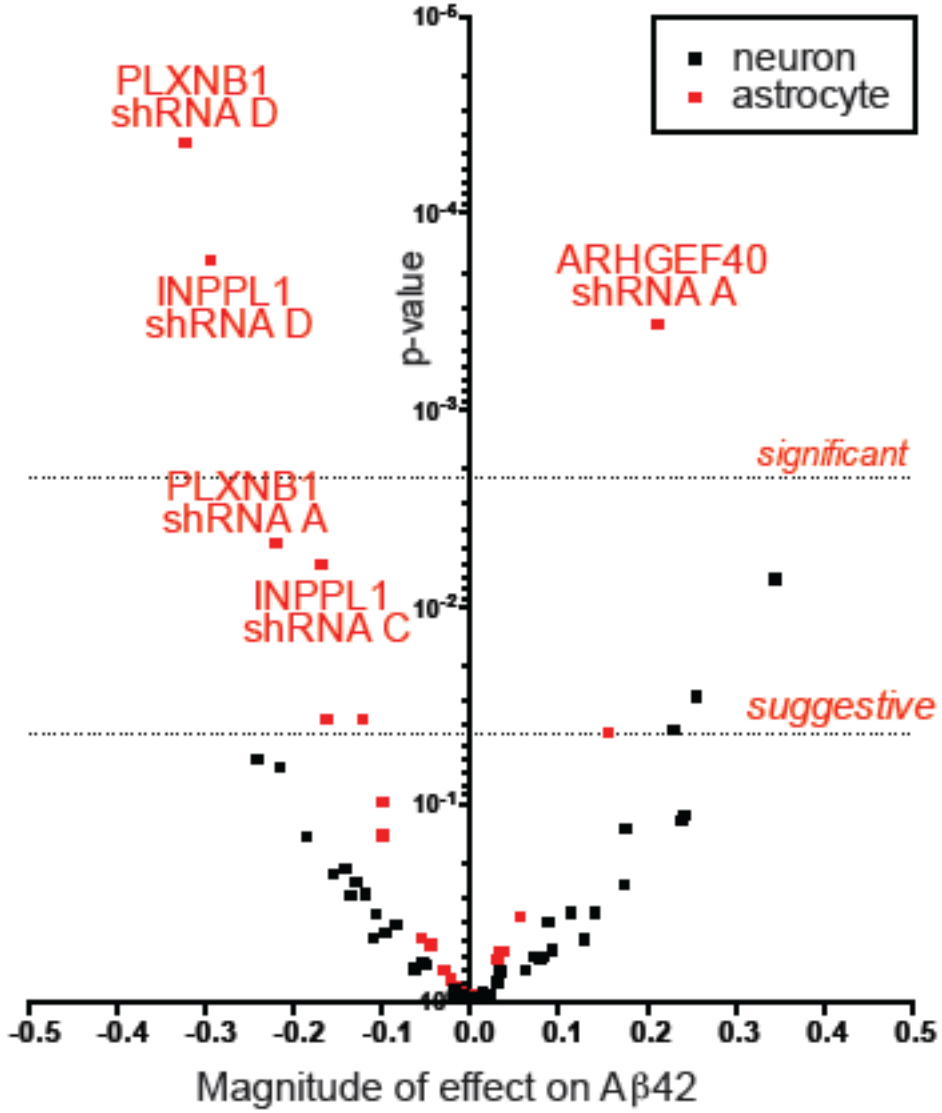
Macrophage/ MDMi

<u>Perturbation</u>
shRNA
CRISPR
400 targets

Assessment
Altered target RNA expression
Altered network
APP secretion
Synaptic function
….

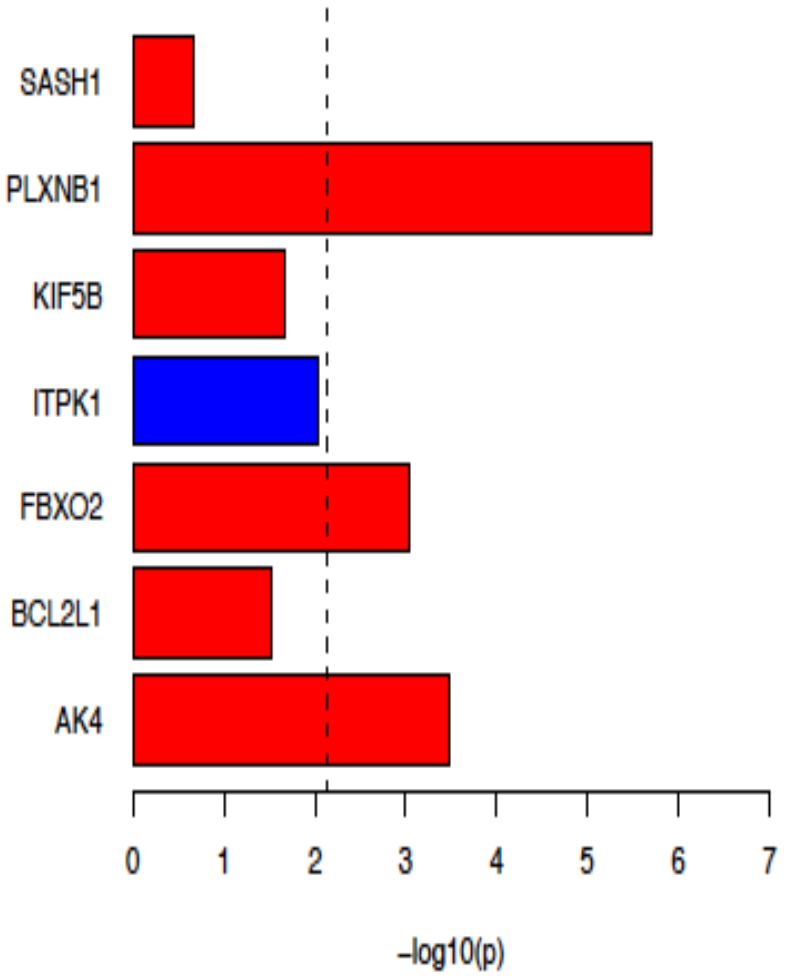
Knockdown results: Astrocytes

- shRNA constructs tested in an in vitro culture system of astrocytes
- Measure effect size and significance of impact of target perturbation on Aβ secretion



Mostafavi*, Gaiteri*, *et al Nat Neuro* 2018

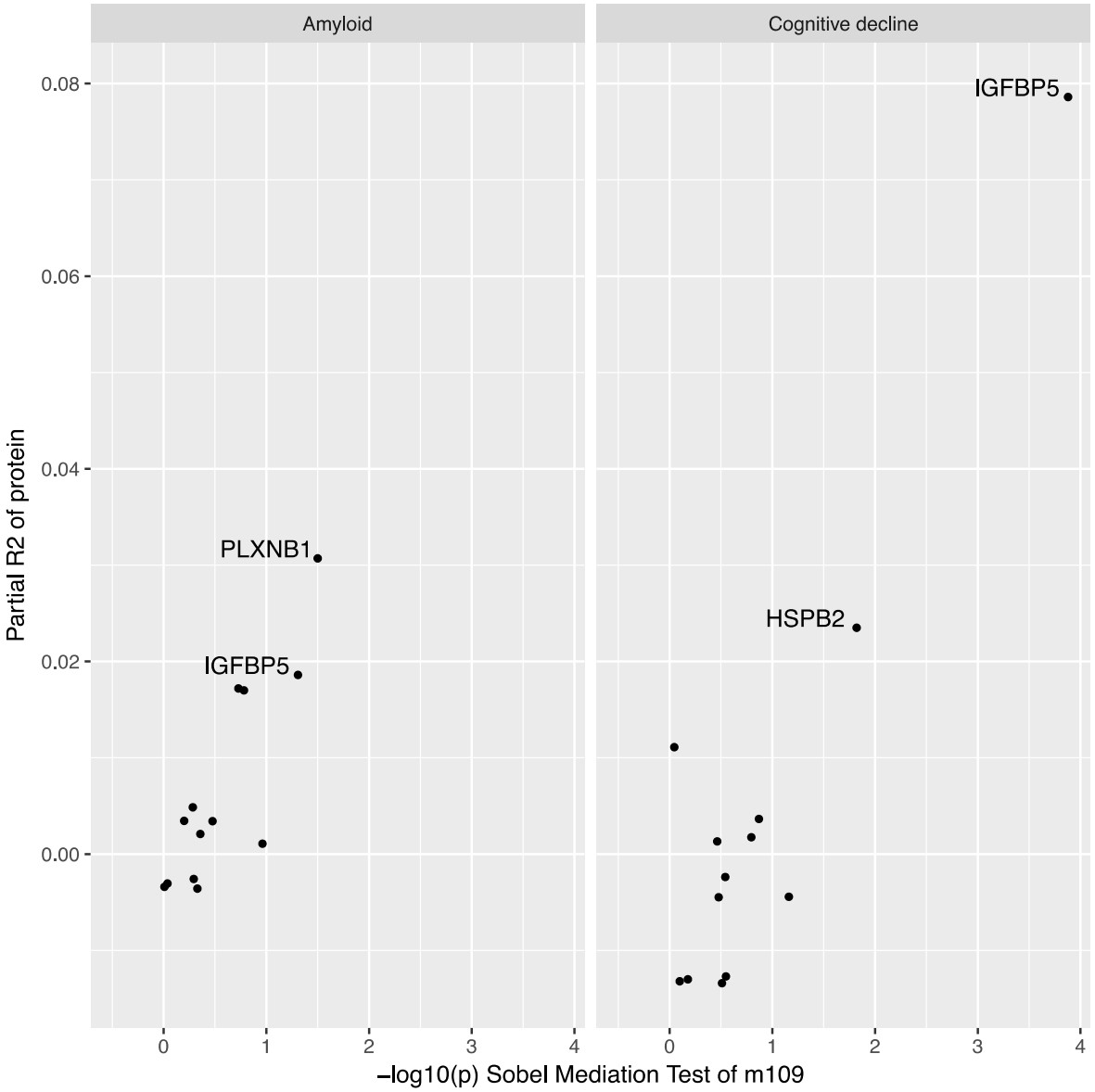
Validation step II: proteomic measures



- Selected m109 genes measured in 800 frontal cortices using targeted SRM proteomics
- At the protein level, cortical PLXNB1 is significantly associated with amyloid deposition
- AK4 and FBXO2 also meet this threshold of significance

Yu *et al Ann Neurol* 2018

Heterogeneity of effect among m109 proteins



13 proteins from m109 were measured in 834 ROSMAP brains

PLXNB1 captures the amyloid effect of m109 well but IGFBP5 may better capture the cognitive decline aspect

IGFBP5 is a binding protein that regulates insulin-like growth factors (IGFs) which plays a crucial role in neurodevelopment and apoptosis. Mouse models show that neuronal IGFBP5 overexpression results in motor neuron degeneration and myelination defects.

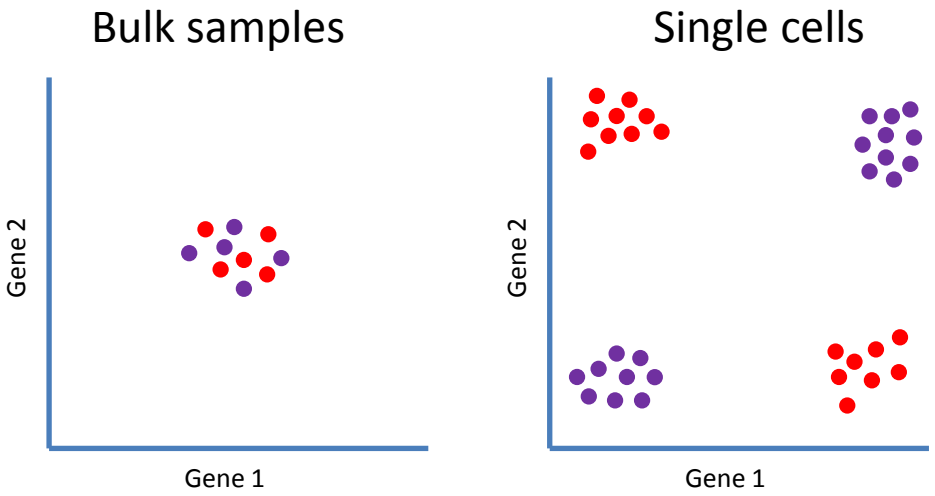
From bulk tissue to single cells

Aims of single-cell analysis

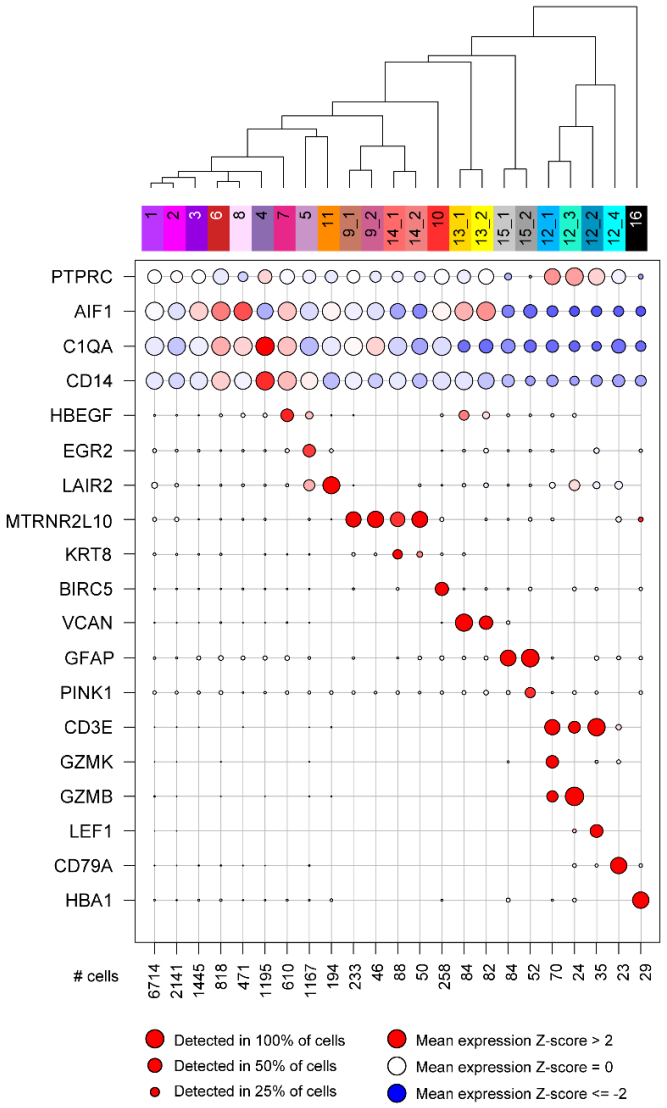
Uncover heterogeneity masked in bulk tissue

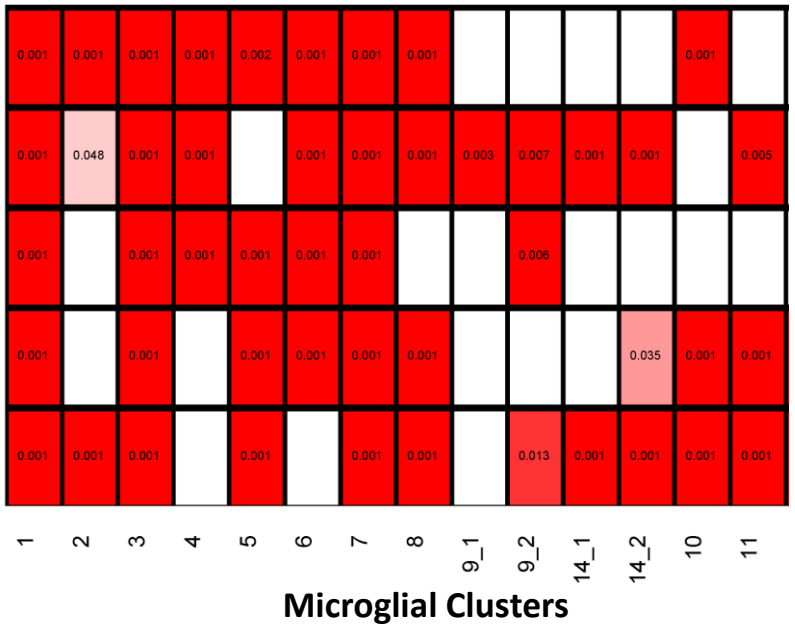
Disentangle cell type proportion from cell type expression changes

Integrate with other cell type-specific profiling (on purified populations, etc.)



Heterogeneity of live microglia from aged brains

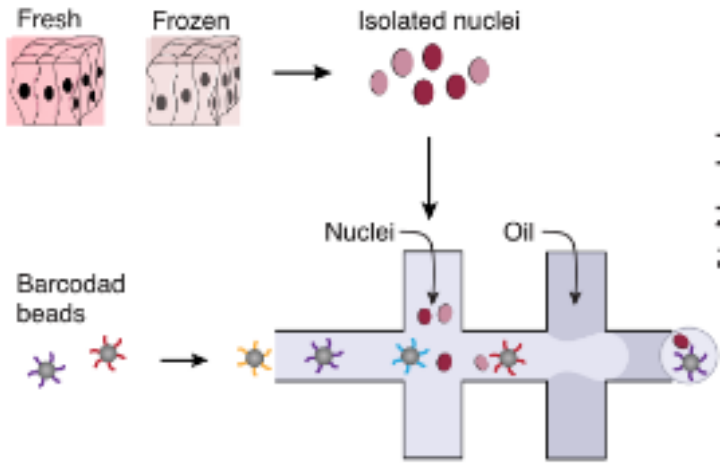


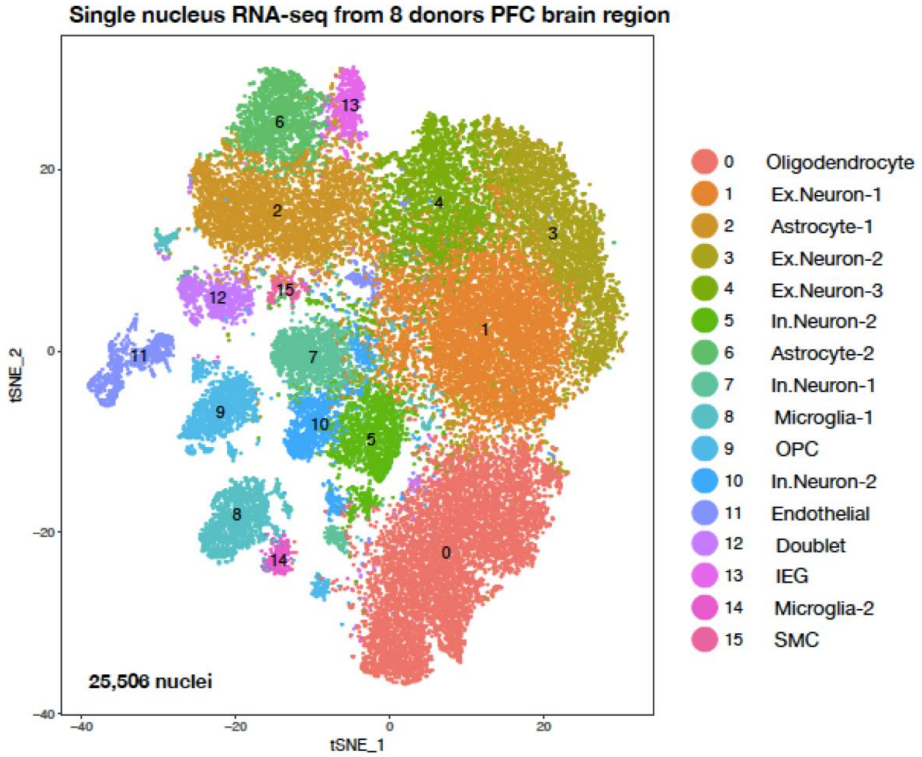


- Each module represents a transcriptional program
- Modules do not correspond to a specific microglial cluster
- May be difficult to infer microglial subsets from cortex-level data

Olah *et al BioRxiv* 2018

Heterogeneity of cell types in single-nucleus RNA-seq from frozen brain tissue





Sampling plan:

DLPFC from ~500 ROSMAP subjects (matched to those with bulk tissue RNA-seq)

~3-7k cells from each sample

Naomi Habib & Cristin McCabe

Summary

- We have established a network map of the aging frontal cortex using tissue-level RNAseq data
- Microglial component of network can be dissected
  - Bulk tissue network may not capture microglia complexity
- Predictions of prioritized genes have been validated *in vitro* and in pathologic material at the protein level
- m109 proteins are heterogeneous in their associations
  - PLXNB1 - strong effect on amyloid accumulation
  - IGFBP5 - stronger effect on cognitive decline

- Currently in progress:
  - Enhance target nomination algorithm
  - Assess functional heterogeneity among coexpressed genes
  - Generate and process large-scale single-nucleus data

Acknowledgements

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Module to trait correlations

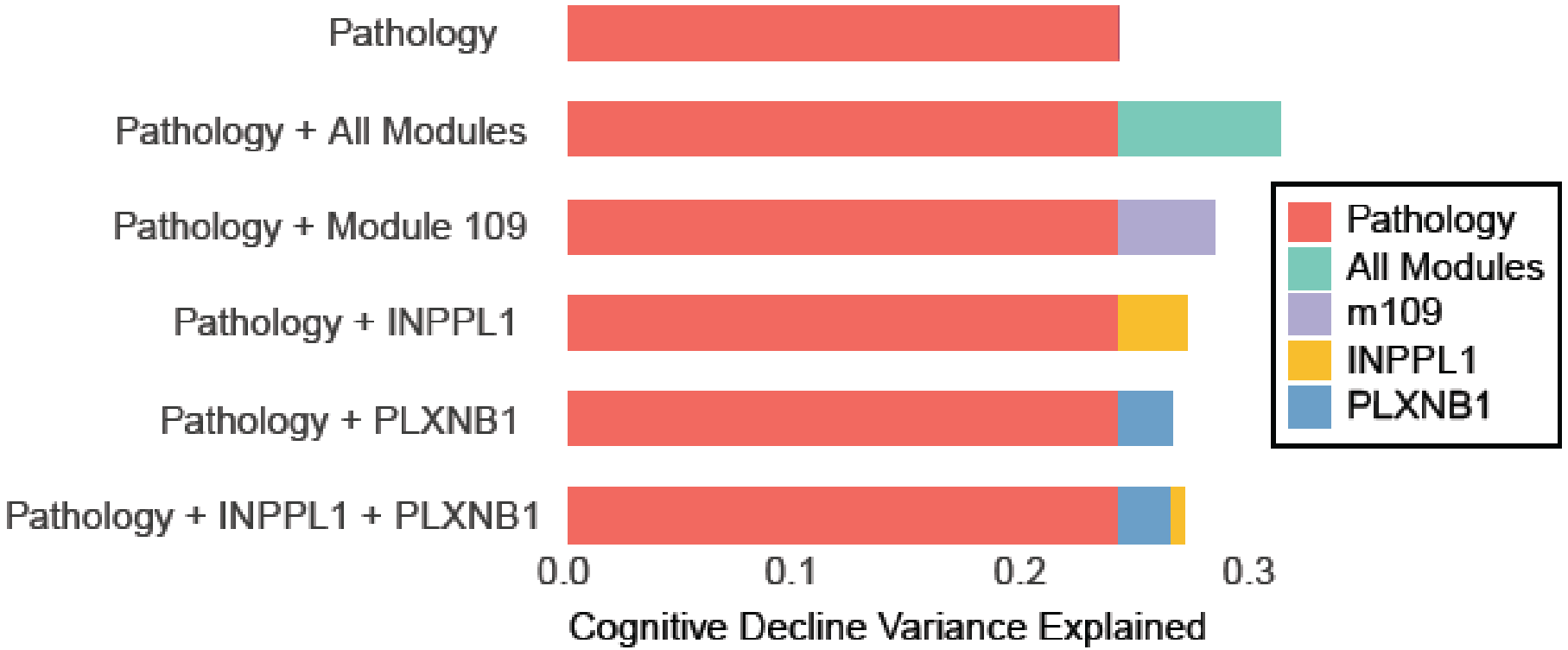
module id	# of genes	representative GO category	Amyloid	Tau	pathoAD	GlobCog Slope	pmAD
9	243	Transcription	0.17223405	1.340278252	0.206256338	0.008911847	0.155643173
10	138	RNA processing	0.22957448	1.200406382	0.024957558	0.122193846	0.176610082
14	347	mitochondrial	4.848962978	0.176614154	1.020376134	2.834063516	1.501505204
16	352	Nueoronal/synapse part	1.520837976	0.274933069	0.049486545	0.202625757	0.116459258
23	251	Nueoronal/synapse part	2.887190918	1.044024213	1.264844337	2.94032218	1.24699196
106	489	mitochondrial matrix	0.369709664	0.456714878	0.103126212	0.181193277	0.316290469
107	416	Fatty acid metabolism/Transmembrane proteins/Neuronal System	1.424826489	0.380349883	0.158000237	0.954024021	0.507623152
109	390	transcription	6.232733137	1.869366774	3.584664339	9.887093208	5.205212621
110	348	Cytoskeleton/motor protein	4.805974065	0.300841472	2.300858879	3.755986452	2.870447473
111	244	transcription	5.252366834	0.29199155	1.622826219	4.090839335	3.428766941
112	64	Cell membrane/signaling peptide/immune	1.354109449	0.033845378	0.263805894	0.678167869	1.097225282
113	313	Metabolism of protein	2.479398982	0.196892875	0.359282893	1.48621899	1.339077684
114	276	Immune response/NF-KappaB	3.524116463	0.936804977	1.55517684	3.736029203	2.144167608
115	232	immune response	1.4404223	0.105790513	0.251757776	1.612195613	0.804028905

Modules:

- Defined using coexpressed genes, with the Speakeasy algorithm
- 47 modules defined with >20 genes each

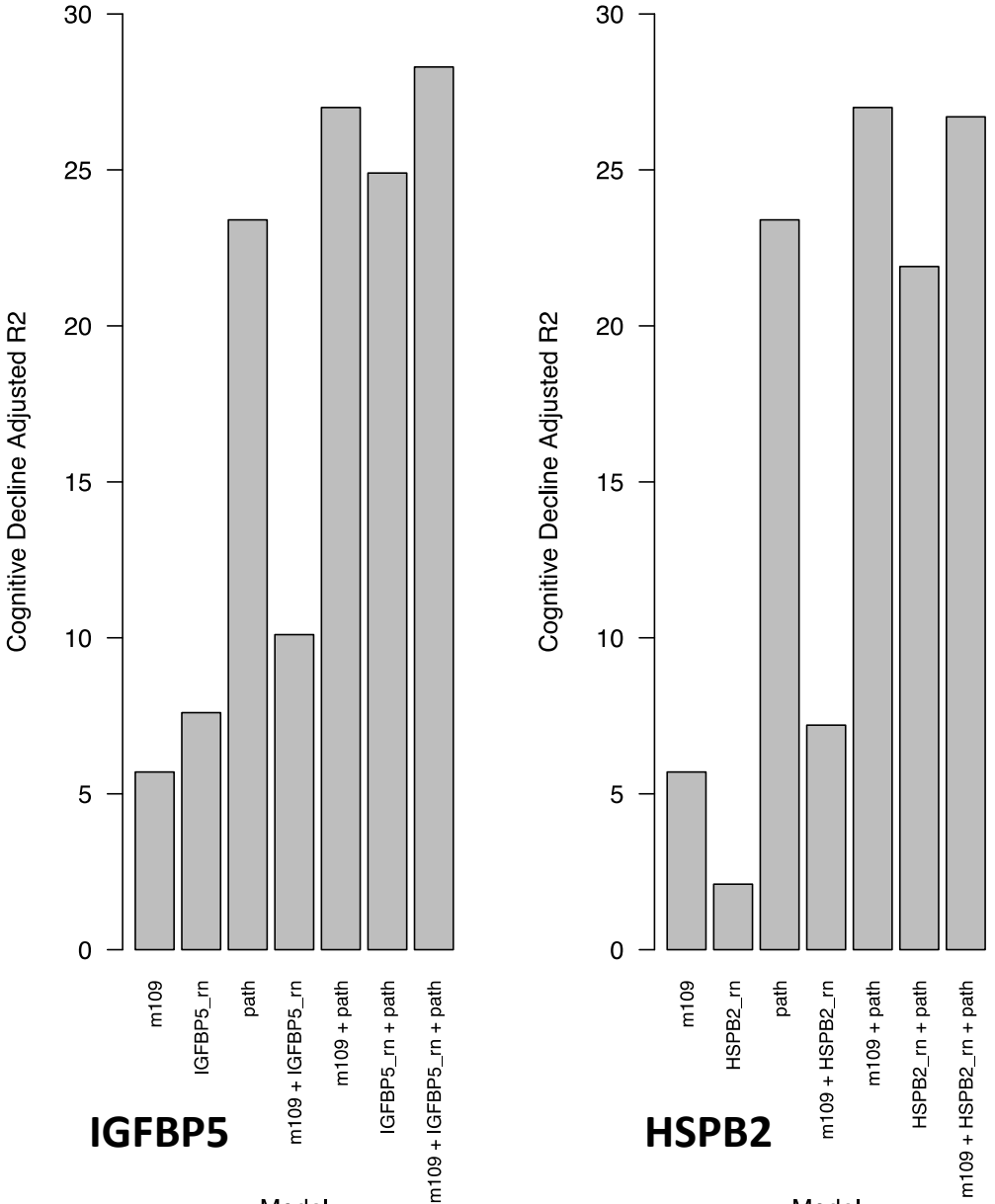
Mostafavi*, Gaiteri*, *et al Nat Neuro* 2018

Variance in cognitive decline explained by m109



Mostafavi *et al*
Nat Neuro 2018

M109 proteins and unexplained variance

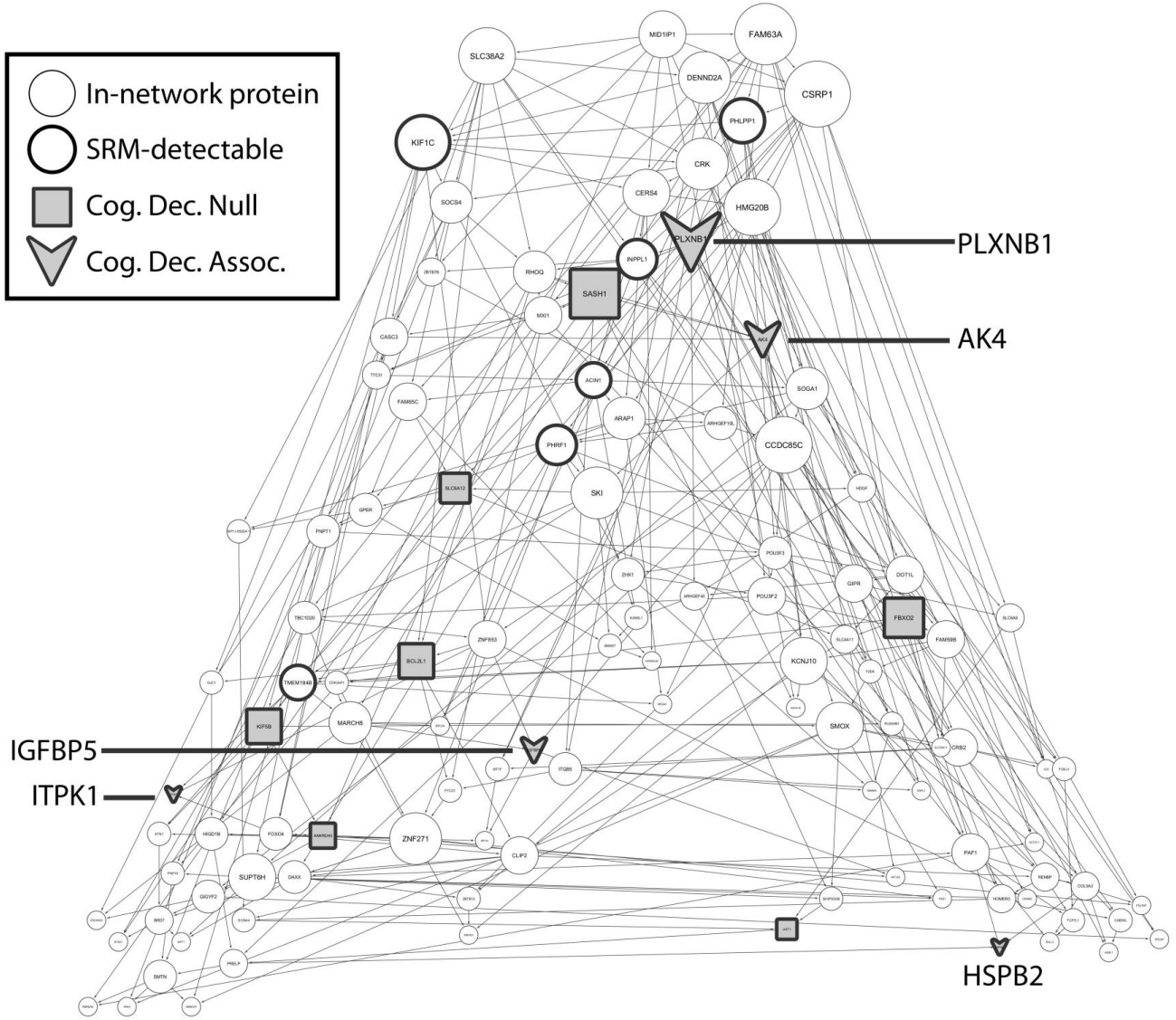


IGFBP5 protein level may capture some variance in cognitive decline not captured by AD pathology or the m109 RNA measure.

Accounting for all 7 neuropathologies, IGFBP5 protein levels explained additional 3% of variance.

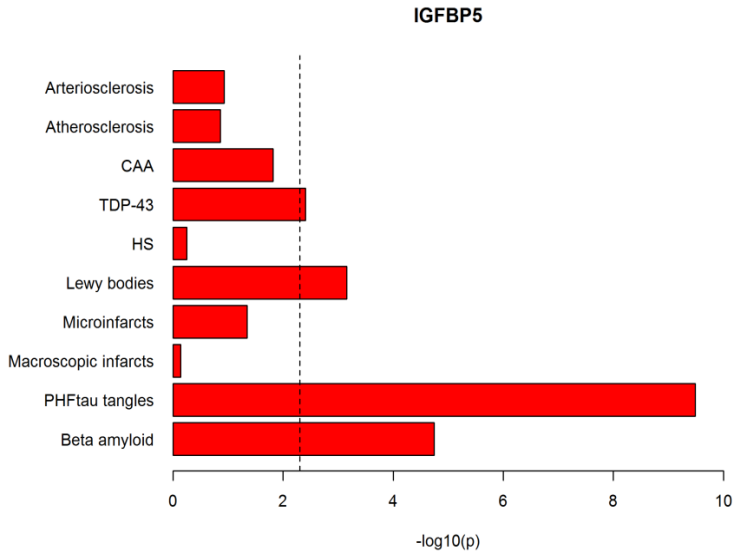
- HSPB2: 0.4%
- AK4: 1%
- Together: 4% of variance in cognitive decline, an important fraction for which we now have biological correlates to explore further.

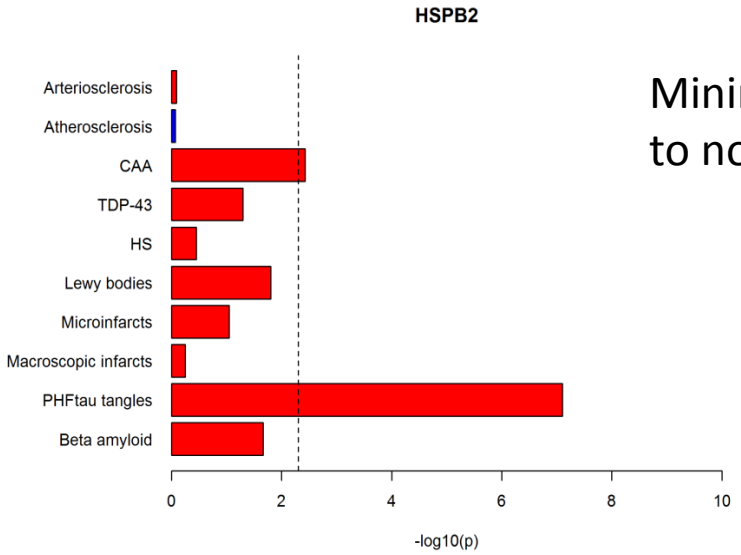
Location of validated proteins in m109 network



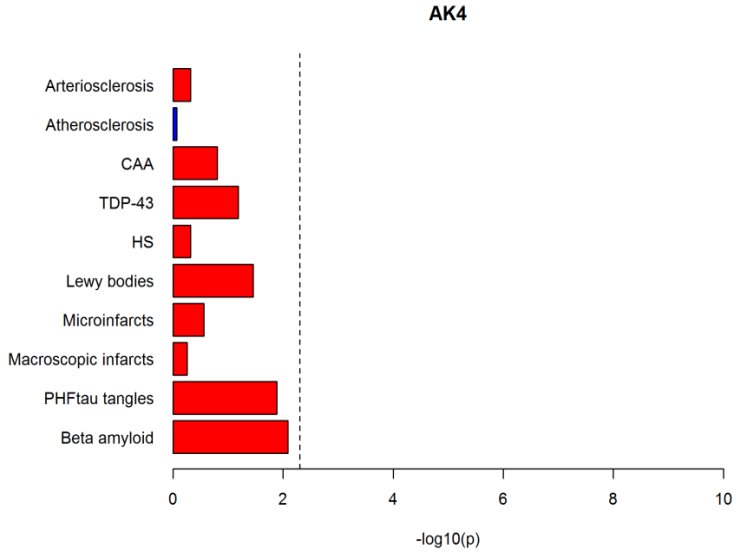
Yu *et al Ann Neurol* 2018

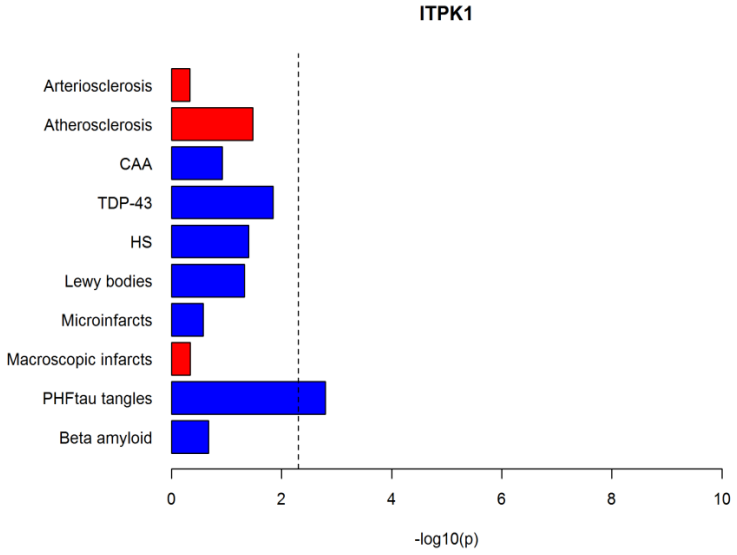
Other m109 proteins





Minimal association to non-AD pathology





Yu *et al Ann Neurol* 2018