MAYO CLINIC

Comparative Multi-omics for Generating New Disease Insights and Novel Target Discovery

Nilüfer Ertekin-Taner, M.D., Ph.D.
Professor of Neurology and Neuroscience
Mayo Clinic Florida

NIA-AA Symposium
Enabling Precision Medicine for Alzheimer's Disease Through Open Science

July 19th, 2018

Acknowledgements

Ertekin-Taner Lab

Mariet Allen*

Minerva Carrasquillo*

Xue Wang*

Thuy Nguyen

Sarah Lincoln

Kim Malphrus

Joseph Reddy

Samantha Strickland

Tulsi Patel

Olivia Conway

Helene Moral

Jez Burgess

Lili N'songo

Michaela Kachadoorian

Joanna Siuda

Morgane Corda

Jenny Bredenburg

Nji Nijkem

Jens Watzlawik

Charlotte Ho

Xin Que

Neuroscience/ Neurology

Steven G. Younkin

Dennis Dickson

Neil Graff-Radford

Ronald C. Petersen

Health Sciences Research

Yan Asmann

Julia Crook

Dan Serie

Research IT

Curt Younkin

Andy Cook

AMP-AD U01

University of Florida

Todd Golde

Jada Lewis

Paramita Chakrabarty

Yona Levites

Institute for Systems Biology

Nathan Price

Cory Funk

Hongdong Li

Tom Beach (Banner)

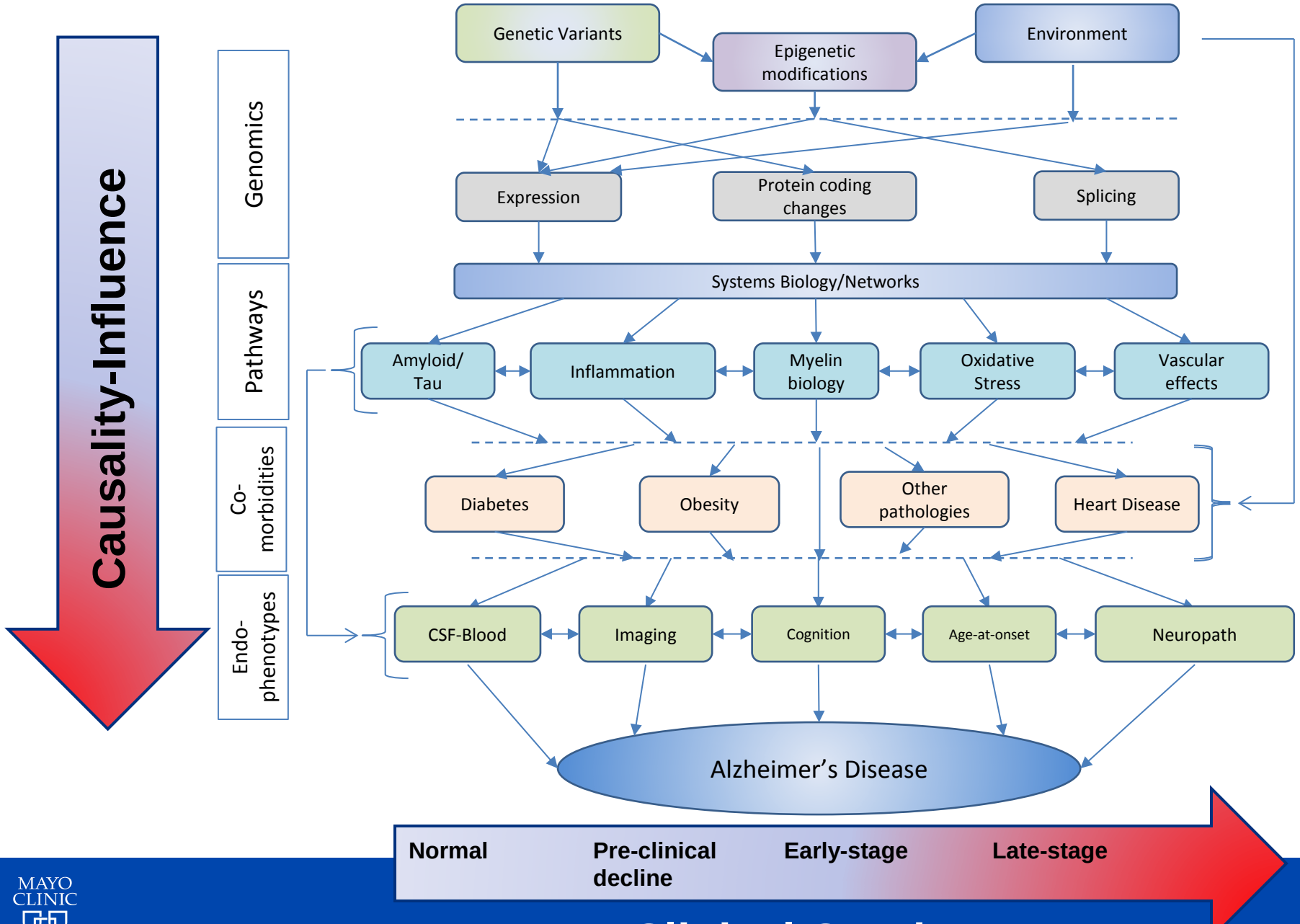
M²OVE-AD RF1

Guojun Bu

Kejal Kantarci

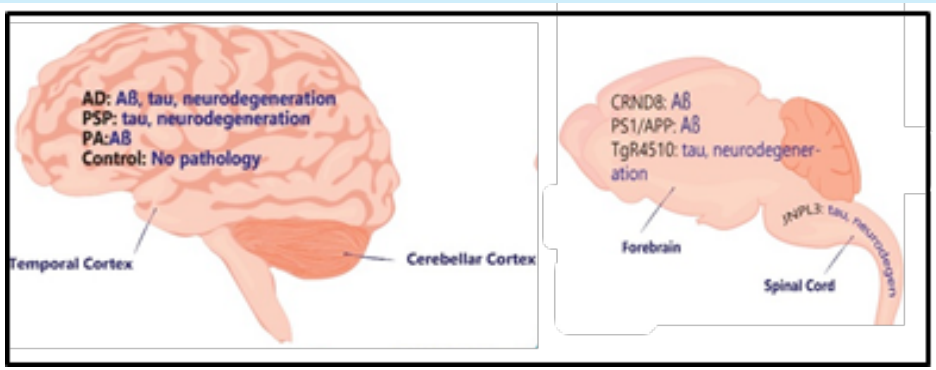
Alfredo Quiñones-Hinojosa

Model: From Genomics to Molecular Mechanisms



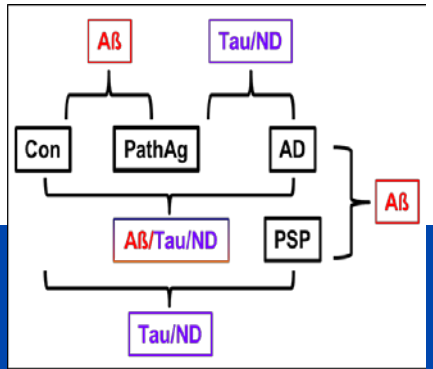
Aims: AMP-AD U01 AG046193

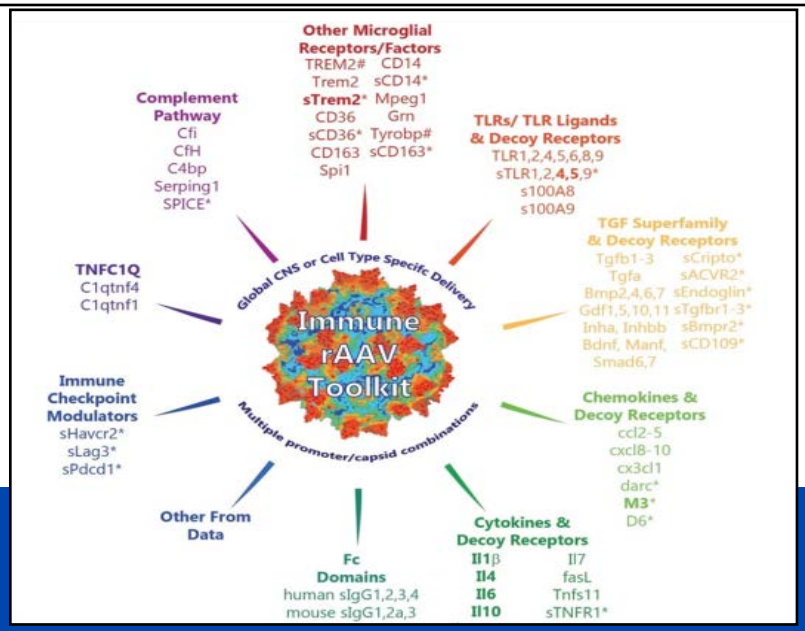
Original Aim 1: To detect transcript alterations in innate immunity genes in mice and humans.	Original Aim 2: To assess AD risk conferred by variants in innate immunity genes from Aim 1.	Original Aim 3: To manipulate innate immune states in vivo.	Original Aim 4: To determine outcome of gene manipulation in wild type mice.
-RNAseq human and mice brains. -Differential expression. -Protein/Nanostring validation -Expression quantitative trait loci (eQTL).	-Test eQTL for effects on AD risk -Functionally annotate AD risk variants for effects on gene expression. -Transcription factor networks.	-rAAV based genetic manipulation in mice and cells. -Evaluate Aß, tau, neurodegeneration outcomes in model systems.	Behavioral studies in nontransgenic mice.



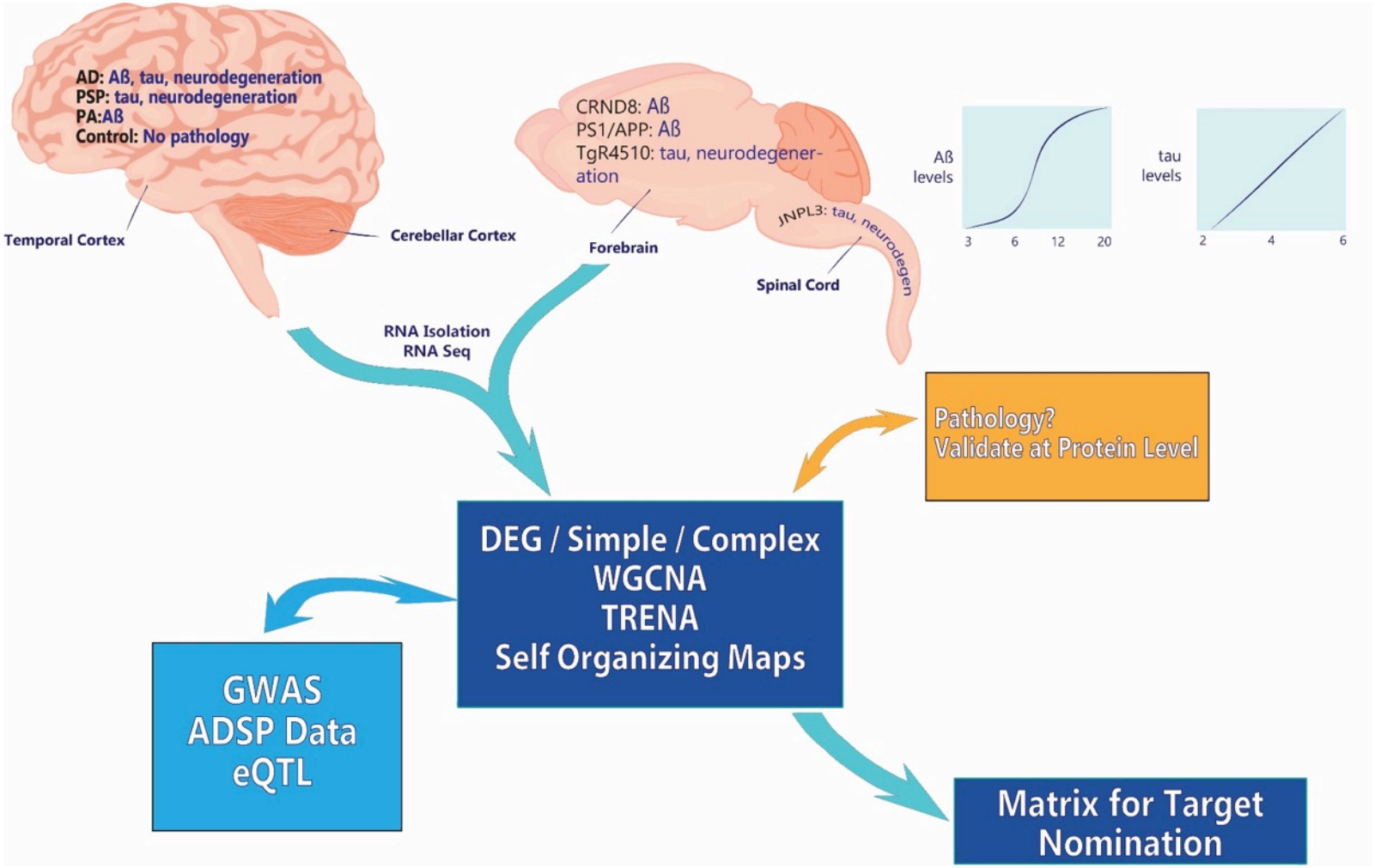
- Pathologic specificity (Tau vs. Amyloid),
- Regional specificity (CER vs. TCX)
- Temporal change (Mouse expression)
- Cell composition dependent vs. not
- Differential expression, intron retention, networks
- Integrative –omics analysis (expression, genetic variants, protein)

Diagnosis	Pathology	
	Amyloid beta	Tau
AD	yes	yes
PSP	no	yes
Pathologic Aging	yes	no
Control	no	no





Approach: Target Discovery



Data Generation

Human RNAseq: n= 555

Tissue Source	Tissue Region	Diagnosis				
		AD	PSP	Path Aging	Control	Total
Mayo Clinic Brain Bank (Dennis Dickson)	TCX	84	84	0	31	199
BannerSunHealth (TomBeach)		0	0	29	49	78
Mayo Clinic Brain Bank (Dennis Dickson)	CER	86	84	0	34	204
BannerSunHealth (TomBeach)		0	0	28	46	74

Mayo Clinic Florida

Mouse RNAseq: n= 172

rTG4510: n=36
P301L: n=24
APPPS1: n=24
CRND8: n=88

University of Florida

Trizol+Qiagen RNeasy Dnase + Agilent QC

Mayo Clinic Medical Genome Facility: TruSeq Library + Illumina HiSeq2000 (101 bp, PE, 3 samples/lane)

Institute for Systems Biology

Mayo Clinic Florida

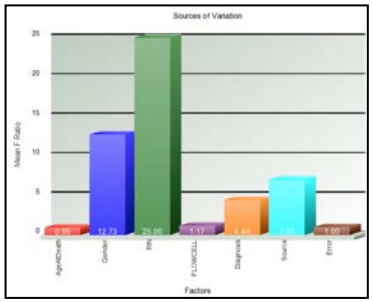
ISB - SNAPR alignment - Filter by Phred scores - normalize to CPM

Mayo – MAPRSeq Pipeline – CQN normalization – Variant calls

Data Analysis

Data Processing

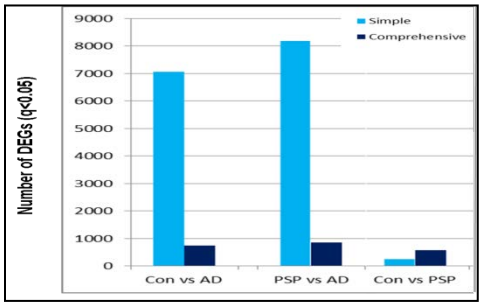
Data QC and sources of variation



Profiling (DEG)

Human and mouse brain transcript profiling

Cell type composition analyses

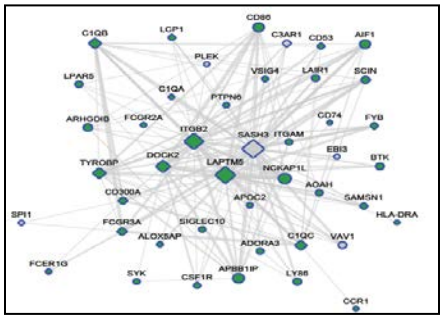


Networks

Co-expression networks

TReNa

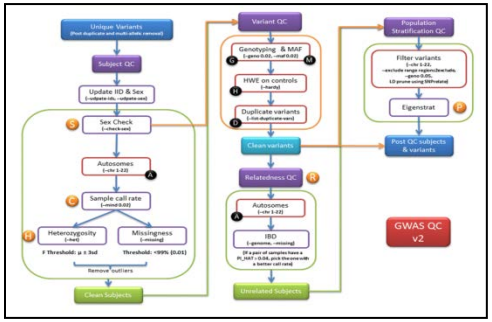
Intron Retention

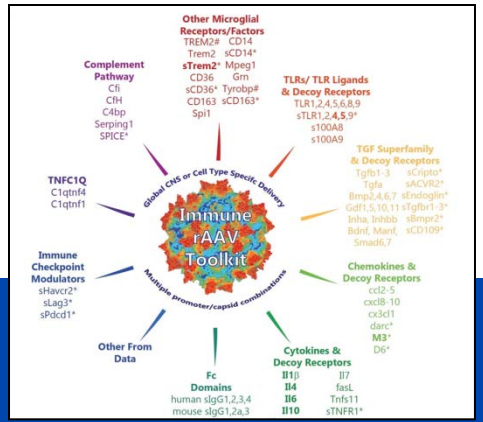


Omics Integration

eQTL

WGS





Human –Omics Data

A. Mayo LOAD GWAS (n=2,099) (Data Citation 2)

Carrasquillo et al., *Nat Gen*, 2009.

2 ante-mortem cohorts: Mayo Clinic Jacksonville (n=684) and Rochester (n=946).

1 post-mortem cohort: Mayo Clinic Brain Bank (n=469)

B. Mayo eGWAS (n=773) (Data Citation 3,4)

Zou et al., *PLoS Genet*, 2012.

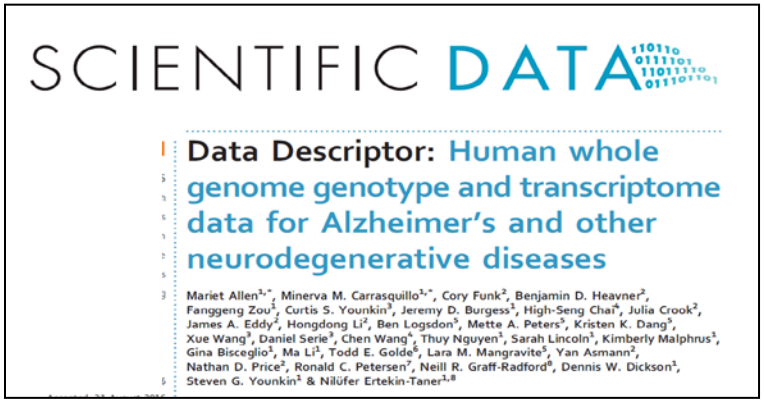
CER samples with WG-DASL gene expression (n=202 AD, 197 non-AD)

TCX samples with WG-DASL gene expression (n=197 AD, 177 non-AD)

C. Mayo Pilot RNAseq (n=94 AD, 92 PSP) (Data Citation 5)

Allen et al., Neurol Genet, 2015; *Acta Neuropath*, 2016.

R01 AG032990
P50 AG0016574
R01 NS080820


SCIENTIFIC DATA

Data Descriptor: Human whole genome genotype and transcriptome data for Alzheimer's and other neurodegenerative diseases

Mariet Allen[1,*], Minerva M. Carrasquillo[1,*], Cory Funk[2], Benjamin D. Heavner[2], Fanggeng Zou[1], Curtis S. Younkin[3], Jeremy D. Burgess[1], High-Seng Chai[4], Julia Crook[2], James A. Eddy[2], Hongdong Li[2], Ben Logsdon[5], Mette A. Peters[3], Kristen K. Dang[3], Xue Wang[3], Daniel Serie[3], Chen Wang[4], Thuy Nguyen[1], Sarah Lincoln[1], Kimberly Malphrus[1], Gina Bisceglio[1], Ma Li[1], Todd E. Golde[6], Lara M. Mangravite[5], Yan Asmann[2], Nathan D. Price[2], Ronald C. Petersen[7], Neill R. Graff-Radford[8], Dennis W. Dickson[1], Steven G. Younkin[1] & Nilüfer Ertekin-Taner[1,8]

- Gene expression on >1,300 brain samples (AD, PSP, controls).
- GWAS genotypes on >2,400.
- WGS on >300.

D. Mayo RNAseq (n=556) (Data Citation 6,7)

CER samples (n=86 AD, 84 PSP, 28 pathologic aging, 80 controls)

TCX samples (n=84 AD, 84 PSP, 30 pathologic aging, 80 controls)

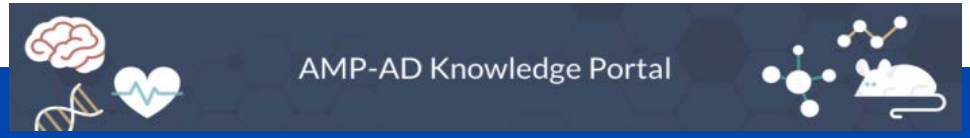
U01 AG046139

Data Deposition

	Directory	Study	Data Type	Tissue	Dx Group	N	Synpase ID
Human	MCADGS	Mayo LOAD GWAS	Genotypes/Covariates	---	AD, Con, nAD	2099	syn3157238
	MCADGS	MayoeGWAS	Array expression/Covariates	CBE	AD, nAD	374	syn3157225
	MCADGS	MayoeGWAS	eQTL results (cis)	CBE	AD, nAD	374	syn3157249
	MCADGS	MayoeGWAS	Array expression/Covariates	TCX	AD, nAD	399	syn3157225
	MCADGS	MayoeGWAS	eQTL results (cis)	TCX	AD, nAD	399	syn3157249
	MCADGS	Mayo Pilot RNAseq	Gene/Transcript counts, Covariates	TCX	AD	96	syn3157268
	MCADGS	Mayo Pilot RNAseq	Gene/Transcript counts, Covariates	TCX	PSP	96	syn3157268
	Mayo RNAseq Study	Cerebellum	Gene/Transcript counts, Covariates	CBE	AD, PSP, PA, Con	276	syn5049298
	Mayo RNAseq Study	Temporal Cortex	Gene/Transcript counts, Covariates	TCX	AD, PSP, PA, Con	275	syn3163039
	Mayo RNAseq Study	Path Aging	Gene/Transcript counts, Covariates	TCX	PA	41	syn7344223
Mouse	Tau and APP ms	APPPS1	Gene/Transcript counts, Covariates	Forebrain	TG/NonTG	40 (various ages)	syn3435792
	Tau and APP ms	TgCRND8	Gene/Transcript counts, Covariates	Forebrain	TG/NonTG	88 (various ages)	syn3435792
	Tau and APP ms	P301L tau (JNPL3)	Gene/Transcript counts, Covariates	Spinal Cord	TG/NonTG	24 (various ages)	syn3157183
	Tau and APP ms	rTg4510	Gene/Transcript counts, Covariates	Forebrain	TG/NonTG	36 (various ages)	syn3157183

Nature Scientific Data

Data uploaded to AMP-AD Knowledge portal on Synapse by the Mayo/UF/ISB team.
MCADGS = Mayo Clinic Alzheimer's Disease Genetics Studies; PA = pathologic aging, Con = Pathology free control; nAD = non Alzheimer's pathology



MAYO CLINIC

Outcomes

- **Conserved brain myelination networks are altered in AD and PSP (*Allen et al., Alzheimer's and Dementia, 2017*). - Comparative -omics, mechanism, novel targets.**
- An intronic variant at the *TREM* locus is associated with higher brain *TREM2* and *TREML1* levels and resides in a TF binding site (*Carrasquillo et al., Alzheimer's and Dementia, 2016*). **- Omics integration, directionality, mechanism.**
- Many AD candidate risk genes have strong eQTL and/or differential expression in brain (*Allen et al, Neurology Genetics 2015, 2017; Ridge et al., Genome Medicine, 2017; Mukherjee et al., Alzheimer's and Dementia, 2017*).
- AD risk genes *PLCG2, ABI3* and *TREM2* have higher levels in AD brains, Aß models and reside in immune networks (*Sims et al., Nature Genetics, 2017*).
- Modulation of innate immunity proteins influences Aß and tau pathophysiology (*Chakrabarty et al., Neuron, 2015; Li et al., FASEB, 2015*).
- Differentially expressed genes/pathways in AD vs. other diagnoses are enriched for immune pathway genes.
- Immunity co-expression networks are enriched for AD risk genes.

Comparative Multi-omics and Convergent Neurodegenerative Disease Mechanisms:

Myelination

Identification of Altered Myelination Networks in AD and PSP:

A Comparative Transcriptome Analysis

ELSEVIER

Alzheimer's & Dementia ■ (2017) 1-15

Alzheimer's & Dementia

Featured Article

Conserved brain myelination networks are altered in Alzheimer's and other neurodegenerative diseases

Mariet Allen[a,1], Xue Wang[b,1], Jeremy D. Burgess[a], Jens Watzlawik[a], Daniel J. Serie[b], Curtis S. Younkin[c], Thuy Nguyen[a], Kimberly G. Malphrus[a], Sarah Lincoln[a], Minerva M. Carrasquillo[a], Charlotte Ho[a], Paramita Chakrabarty[d], Samantha Strickland[a], Melissa E. Murray[a], Vivek Swarup[e], Daniel H. Geschwind[e], Nicholas T. Seyfried[f,g], Eric B. Dammer[f], James J. Lah[g], Allan I. Levey[g], Todd E. Golde[d], Cory Funk[h], Hongdong Li[h], Nathan D. Price[h], Ronald C. Petersen[i], Neill R. Graff-Radford[j], Steven G. Younkin[a], Dennis W. Dickson[a], Julia R. Crook[b], Yan W. Asmann[b], Nilüfer Ertekin-Taner[a,j,*]

Comparative Transcriptome Analysis: Rationale

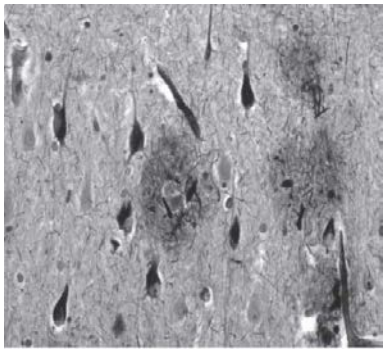
Comparative transcriptome analysis of distinct neurodegenerative diseases can uncover disease pathways that are *unique* or *common* to these diseases.

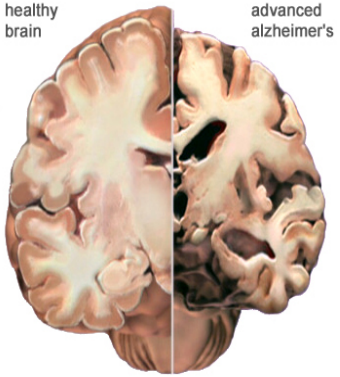
- AD ≠ PSP AND [(ΔAD vs. Con) ≠ (ΔPSP vs.Con)] → **uniquely perturbed pathways**
- [(ΔAD vs. Con) ~ (ΔPSP vs. Con)] → **commonly perturbed pathways**

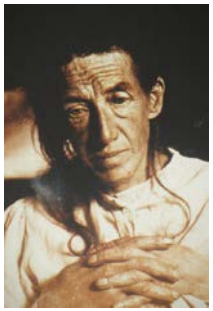
Diagnostic Groups

Diagnosis	Pathology	
	Amyloid beta	Tau
AD	yes	yes
PSP	no	yes

Alzheimer's Disease (AD)



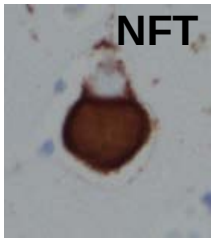


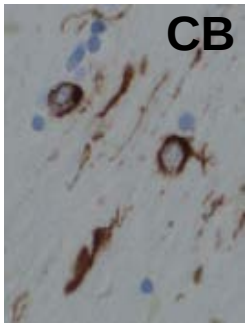


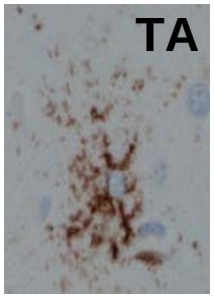
- **Plaques (Aβ)+Tangles (tau).**
- **Dementia: Memory, language, others.**

  - *APP, PSEN1, PSEN2*
  - *APOE ε4*
  - 20 GWAS loci genes
  - *TREM2, PLD3*

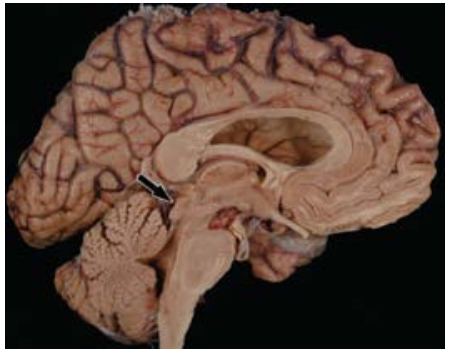
Progressive Supranuclear Palsy (PSP)







(Dickson et al, 2007)



- **Tangles+ tau-positive glial lesions.**
- **Parkinsonian disorder: Falls, eye movement.**

  - *MAPT (H1 haplotype)*
  - *6* other GWAS loci (*MOBP* etc.)

Comparative Transcriptome Analysis: Approach

Discovery and Replication Cohorts

	Mayo Clinic eGWAS (WG-DASL)				Mayo Clinic RNAseq		
Transcriptome profiling	**Temporal Cortex (TCX)**		**Cerebellum (CER)**		**Temporal Cortex (TCX)**		
	AD	**PSP**	**AD**	**PSP**	**AD**	**PSP**	**Control**
N	181	97	173	96	80	82	76
Females (%)	94 (52%)	40 (42%)	88 (51%)	37 (39%)	49 (61%)	33 (40%)	38 (50%)
Age: Mean (SD)	74 (5.6)	72 (5.3)	73 (5.7)	72 (5.0)	83 (8.6)	74 (6.5)	84 (9.3)
RIN: Mean (SD)	6.3 (0.8)	7.0 (1.0)	7.1 (1.0)	7.1 (1.0)	8.6 (0.6)	8.5 (0.5)	7.6 (1.0)

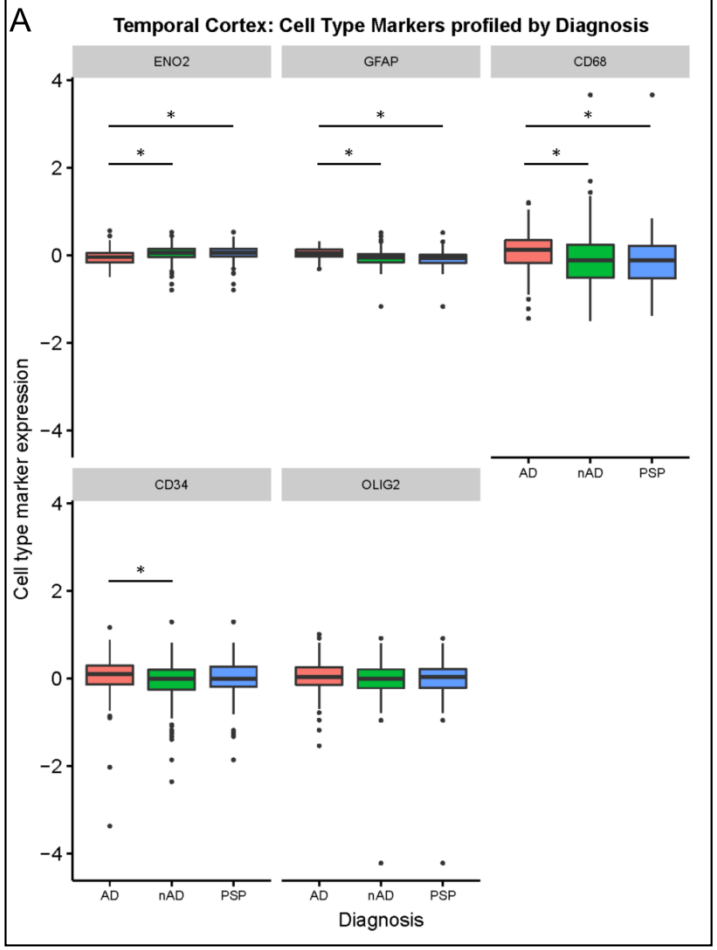
Transcriptome Profiling: Multi-variable linear regression analysis in R controlled for covariates (age, sex, RIN, APOE, plate for discovery; age, sex, RIN, tissue source, flowcell for replication cohort analyses).

Network Analysis: Weighted Gene Co-expression Network Analysis (Langfelder&Horvath BMC Bioinform, 2008). Gene expression residuals after accounting for covariates.

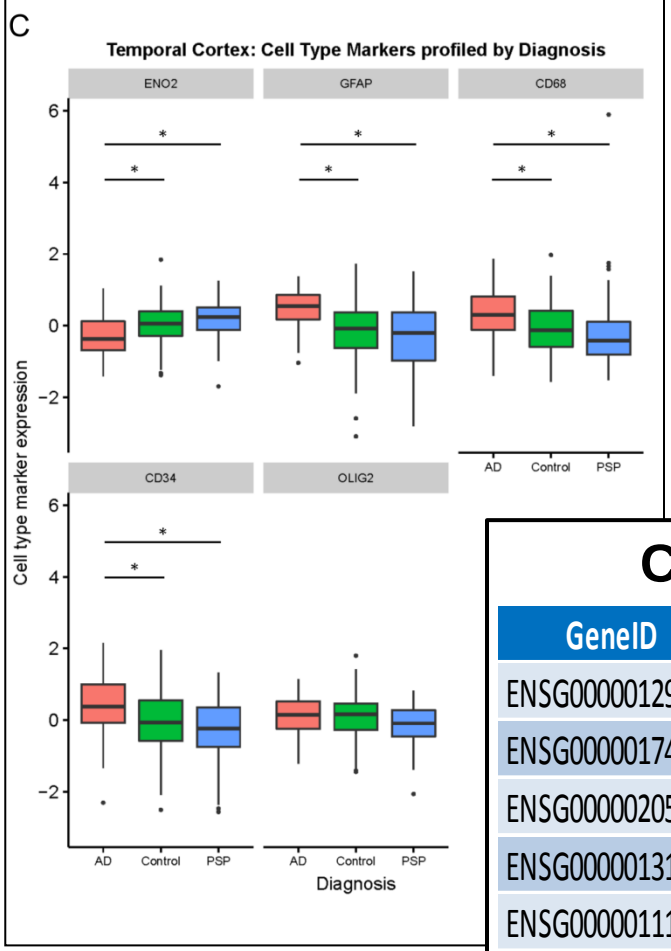
Goal: Discover common and distinct dysregulated expression networks and key molecules that underlie disease pathways in AD and PSP.

Comparative Transcriptome Analysis:
Cell Type Adjustment

Discovery Cohort



Replication Cohort



Covariates included

- Age at death
- Gender
- RIN
- Source
- Flowcell/plate
- Celltype markers

Simple Model

Comprehensive Model

Cell type markers

GeneID	GeneName	Chr	Celltype
ENSG00000129226	CD68	chr17	Microglial
ENSG00000174059	CD34	chr1	Endothelial
ENSG00000205927	OLIG2	chr21	Oligodendroglial
ENSG00000131095	GFAP	chr17	Astrocytic
ENSG00000111674	ENO2	chr12	Neuronal

Myelination Networks Are Up In AD vs. PSP Temporal Cortex (TCX) – Discovery Cohort

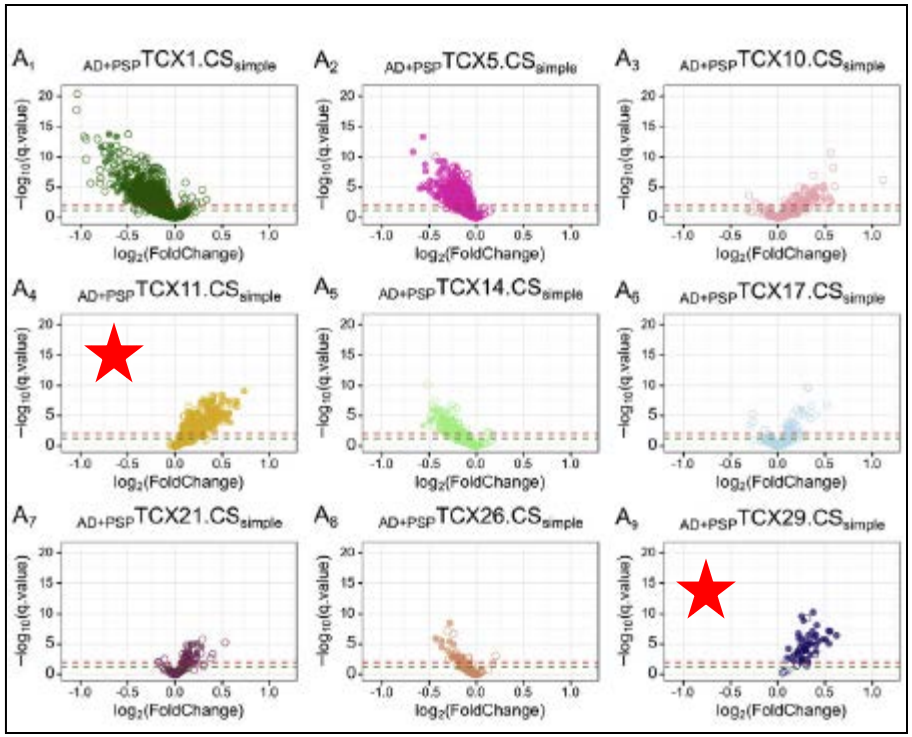
Table 1
Temporal cortex coexpression networks in the discovery cohort with significant cell type enrichment

			Cell type enrichment			Disease association		Top GO biological process		
Model	Module name	Module size	Cell type	OR	*P* Value	Beta	*P* Value	ID	Name	Enrichment *P* value
Simple	$_{AD+PSP}$TCX17.CS$_{simple}$	213	Astrocyte	54.4	5.83×10^{-80}	0.06	3.34×10^{-1}	GO:0007399	Nervous system development	3.25×10^{-6}
	$_{AD+PSP}$TCX10.CS$_{simple}$	404	Microglia	55.6	7.34×10^{-158}	0.11	7.66×10^{-2}	GO:0006955	Immune response	1.98×10^{-53}
	$_{AD+PSP}$TCX21.CS$_{simple}$	153	Microglia	4.3	8.74×10^{-3}	0.12	5.09×10^{-2}	NA	NA	NA
	$_{AD+PSP}$TCX1.CS$_{simple}$	2046	Neuron	9.8	1.00×10^{-100}	**−0.21**	$\mathbf{5.50 \times 10^{-4}}$	GO:0007268	Synaptic transmission	2.15×10^{-60}
	$_{AD+PSP}$TCX14.CS$_{simple}$	314	Neuron	9.5	9.06×10^{-27}	**−0.16**	$\mathbf{8.50 \times 10^{-3}}$	GO:0007268	Synaptic transmission	2.93×10^{-12}
	$_{AD+PSP}$TCX5.CS$_{simple}$	654	Neuron	4.9	1.06×10^{-19}	**−0.29**	$\mathbf{1.04 \times 10^{-6}}$	NA	NA	NA
	$_{AD+PSP}$TCX26.CS$_{simple}$	102	Neuron	7.4	2.56×10^{-6}	**−0.14**	$\mathbf{1.96 \times 10^{-2}}$	GO:0098655	Cation transmembrane transport	3.55×10^{-2}
	$_{AD+PSP}$TCX11.CS$_{simple}$	340	Oligodendrocyte	96.4	2.78×10^{-81}	**0.27**	$\mathbf{5.58 \times 10^{-6}}$	GO:0042552	Myelination	1.03×10^{-7}
	$_{AD+PSP}$TCX29.CS$_{simple}$	58	Oligodendrocyte	47.1	2.01×10^{-13}	**0.32**	$\mathbf{4.43 \times 10^{-8}}$	NA	NA	NA
Comprehensive	$_{AD+PSP}$TCX14.CS	264	Astrocyte	31.5	1.86×10^{-59}	−0.11	6.41×10^{-2}	GO:0007399	Nervous system development	6.32×10^{-7}
	$_{AD+PSP}$TCX26.CS	120	Microglia	153.6	9.19×10^{-106}	**−0.17**	$\mathbf{4.56 \times 10^{-3}}$	GO:0006955	Immune response	2.72×10^{-34}
	$_{AD+PSP}$TCX42.CS	41	Neuron	8.9	2.58×10^{-3}	**0.12**	$\mathbf{4.38 \times 10^{-2}}$	NA	NA	NA
	$_{AD+PSP}$TCX27.CS	111	Neuron	10.3	8.18×10^{-12}	−0.01	8.31×10^{-1}	GO:0007268	Synaptic transmission	1.33×10^{-2}
	$_{AD+PSP}$TCX16.CS	219	Neuron	6.7	7.15×10^{-13}	0.01	9.12×10^{-1}	NA	NA	NA
	$_{AD+PSP}$TCX12.CS	305	Neuron	6.6	7.31×10^{-16}	0.02	7.32×10^{-1}	GO:0007268	Synaptic transmission	1.57×10^{-13}
	$_{AD+PSP}$TCX8.CS	377	Neuron	7.1	5.76×10^{-22}	0.03	6.26×10^{-1}	GO:0007268	Synaptic transmission	1.51×10^{-17}
	$_{AD+PSP}$TCX2.CS	752	Neuron	14.1	4.96×10^{-93}	0.08	1.97×10^{-1}	GO:0007268	Synaptic transmission	4.44×10^{-20}
	$_{AD+PSP}$TCX41.CS	41	Oligodendrocyte	40.6	3.98×10^{-8}	0.06	3.02×10^{-1}	NA	NA	NA
	$_{AD+PSP}$TCX40.CS	44	Oligodendrocyte	20.1	2.29×10^{-3}	**0.20**	$\mathbf{8.00 \times 10^{-4}}$	NA	NA	NA
	$_{AD+PSP}$TCX10.CS	308	Oligodendrocyte	81.6	1.70×10^{-72}	**0.19**	$\mathbf{1.43 \times 10^{-3}}$	GO:0042552	Myelination	2.37×10^{-9}

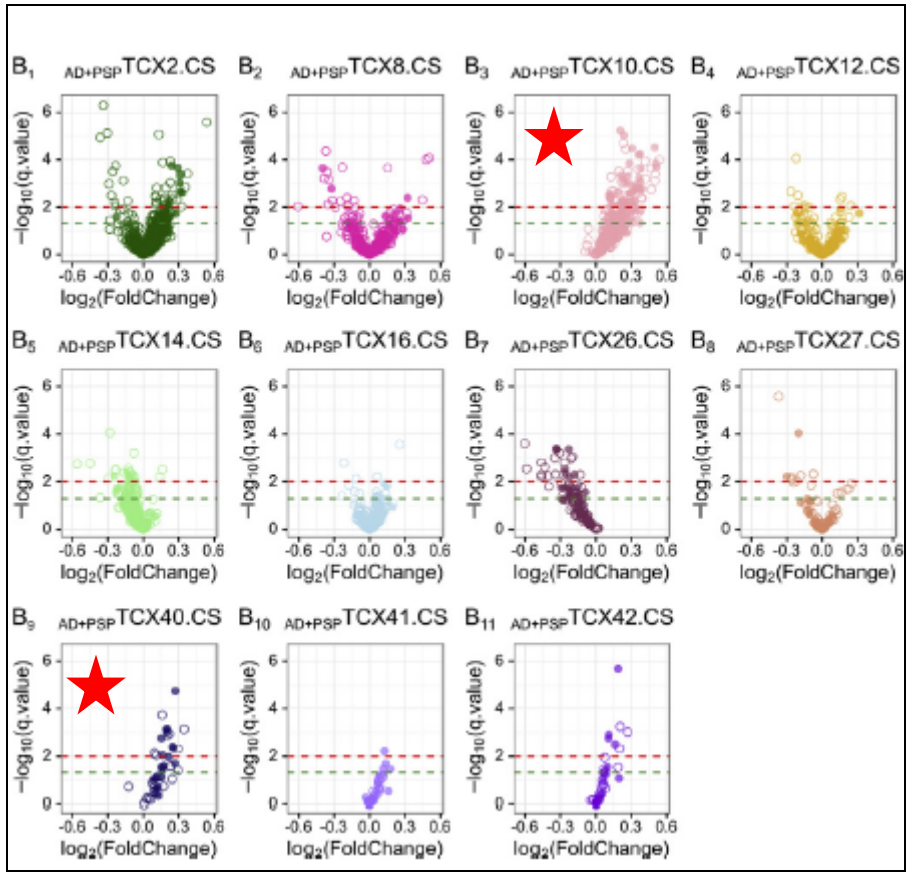
- TCX co-expression networks enriched for oligodendrocyte transcripts and myelination related biological processes are higher in AD vs. PSP.
- This association persists even when adjusting for five CNS cell-specific transcripts (surrogate for cell type composition).

Myelination Networks Are Up In AD vs. PSP Temporal Cortex (TCX) – Discovery Cohort

Simple Model



Comprehensive Model



Myelination Networks Are Up In AD vs. PSP Down in PSP vs. Control Down in AD vs. Control Temporal Cortex (TCX) – Replication Cohort

Table 3
Temporal cortex coexpression networks in replication cohort with significant oligodendrocyte-specific gene enrichment

Model	Diagnostic comparison	Module name	Module size	Number of oligodendrocyte genes in module	Oligodendrocyte enrichment OR	Oligodendrocyte enrichment *P* value	Disease association beta	Disease association *P* value
Simple	AD + Con	$_{AD+Con}$TCX10.CSRS$_{simple}$	398	15	5.95	2.45×10^{-7}	−0.094	9.19×10^{-1}
		$_{AD+Con}$TCX4.CSRS$_{simple}$	924	73	40.48	2.44×10^{-63}	−0.008	2.44×10^{-1}
	AD + PSP	$_{AD+PSP}$TCX3.CSRS$_{simple}$	1542	93	125.11	6.12×10^{-80}	0.279	$\mathbf{3.31 \times 10^{-4}}$
	PSP + Con	$_{PSP+Con}$TCX5.CSRS$_{simple}$	737	73	52.71	9.60×10^{-69}	−0.221	$\mathbf{5.19 \times 10^{-3}}$
		$_{PSP+Con}$TCX12.CSRS$_{simple}$	253	15	9.68	5.35×10^{-8}	−0.176	$\mathbf{2.74 \times 10^{-2}}$

- TCX myelination network expression is replicably higher in AD vs. PSP.
- This appears to be due to greater reduction in myelination network gene levels in ‘PSP vs. Control’ than ‘AD vs. Control’.

Myelination Networks Are Up In AD vs. PSP

Down in PSP vs. Control

Down in AD vs. Control

Temporal Cortex (TCX) – Replication Cohort

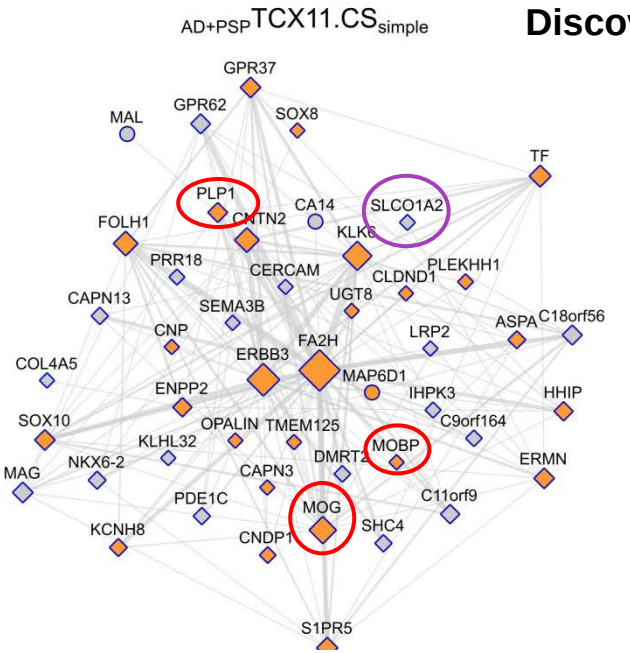
Table 3
Temporal cortex coexpression networks in replication cohort with significant oligodendrocyte-specific gene enrichment

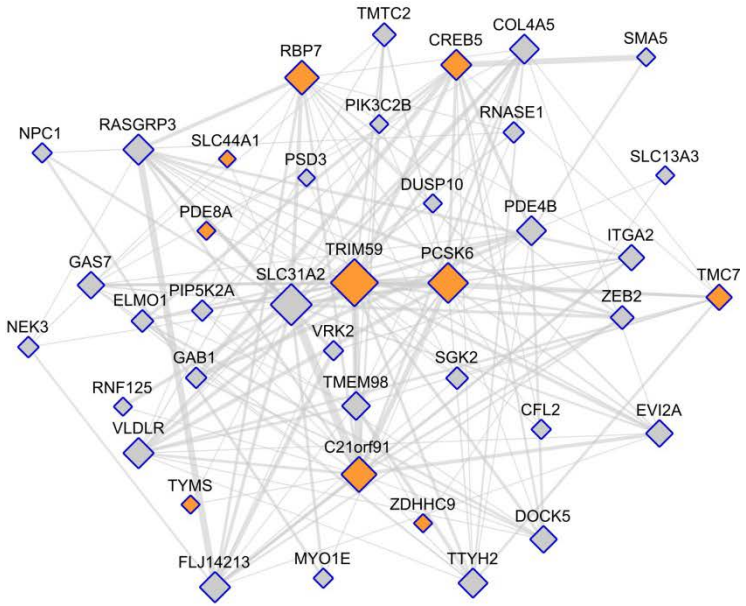
Model	Diagnostic comparison	Module name	Module size	Number of oligodendrocyte genes in module	Oligodendrocyte enrichment OR	Oligodendrocyte enrichment *P* value	Disease association beta	Disease association *P* value
Simple	AD + Con	$_{AD+Con}$TCX10.CSRS$_{simple}$	398	15	5.95	2.45×10^{-7}	−0.094	9.19×10^{-1}
		$_{AD+Con}$TCX4.CSRS$_{simple}$	924	73	40.48	2.44×10^{-63}	−0.008	2.44×10^{-1}
	AD + PSP	$_{AD+PSP}$TCX3.CSRS$_{simple}$	1542	93	125.11	6.12×10^{-80}	0.279	$\mathbf{3.31 \times 10^{-4}}$
	PSP + Con	$_{PSP+Con}$TCX5.CSRS$_{simple}$	737	73	52.71	9.60×10^{-69}	−0.221	$\mathbf{5.19 \times 10^{-3}}$
		$_{PSP+Con}$TCX12.CSRS$_{simple}$	253	15	9.68	5.35×10^{-8}	−0.176	$\mathbf{2.74 \times 10^{-2}}$
Comprehensive	AD + Con	$_{AD+Con}$TCX7.CSRS	526	17	5.15	4.49×10^{-5}	−0.228	$\mathbf{4.12 \times 10^{-3}}$
		$_{AD+Con}$TCX24.CSRS	65	15	46.61	9.42×10^{-17}	−0.143	$\mathit{7.40 \times 10^{-2}}$
		$_{AD+Con}$TCX26.CSRS	52	5	14.81	6.03×10^{-3}	−0.025	7.54×10^{-1}
		$_{AD+Con}$TCX2.CSRS	886	56	19.35	5.81×10^{-38}	−0.042	6.05×10^{-1}
	AD + PSP	$_{AD+PSP}$TCX2.CSRS	946	49	13.44	4.62×10^{-28}	0.009	9.07×10^{-1}
		$_{AD+PSP}$TCX8.CSRS	628	25	7.02	5.42×10^{-10}	0.003	9.66×10^{-1}
		$_{AD+PSP}$TCX26.CSRS	69	15	43.23	2.84×10^{-16}	−0.050	5.29×10^{-1}
	PSP + Con	$_{PSP+Con}$TCX2.CSRS	1291	74	29.01	5.46×10^{-52}	−0.100	2.12×10^{-1}
		$_{PSP+Con}$TCX22.CSRS	112	14	21.9	1.35×10^{-11}	−0.064	4.26×10^{-1}

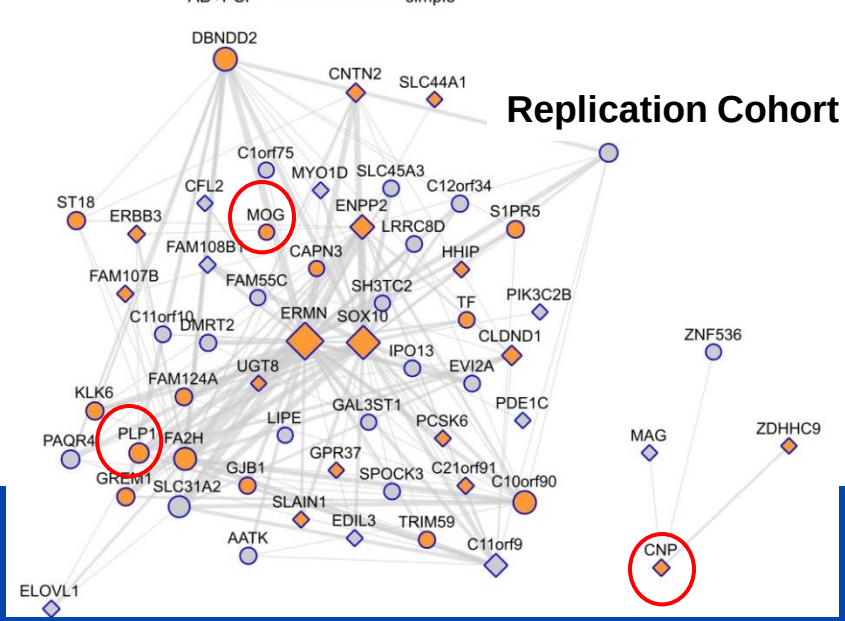
- In comprehensive model for Replication Cohort, trends remain the same but significance reduced for some comparisons (over-correction?).

Myelination Networks Harbor AD and PSP Risk

Discovery Cohort







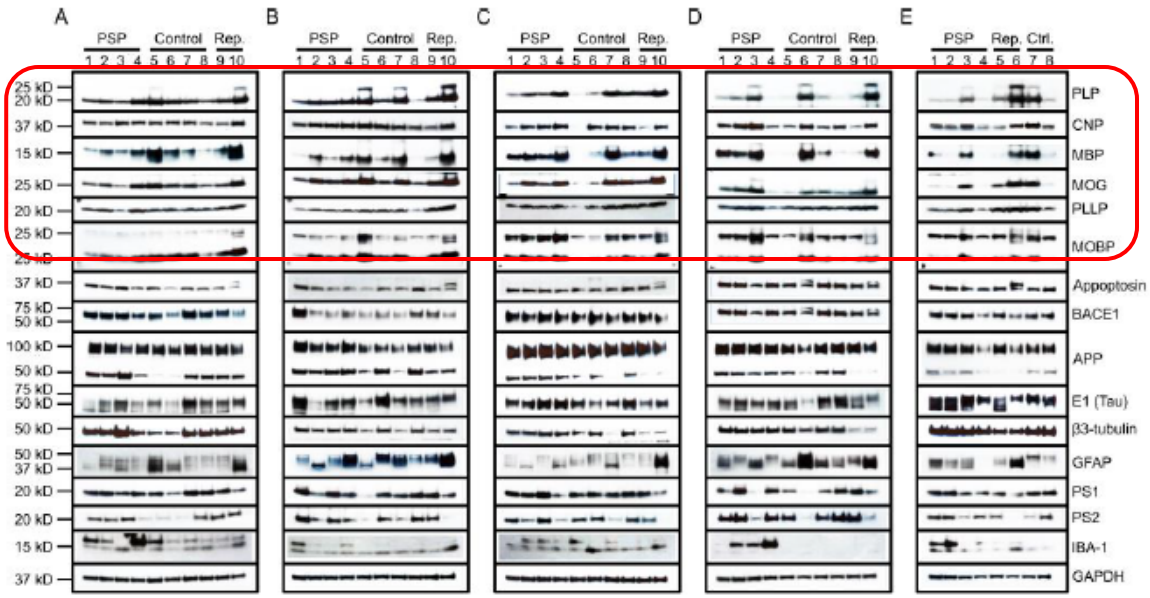
Replication Cohort

Myelination genes:
MOG, PLP1, PLLP, CNP, MOBP

PSP risk genes:
SLCO1A2, MOBP

AD risk/related genes:
BACE1, PSEN1, BIN1, CR1

Myelination Network Perturbations Are Validated at Protein Level



PSP vs. Control					
		Mean ± SD			
Protein	Gene	PSP	Controls	β PSP	p value
PLP	*PLP1*	0.758 ± 0.355	0.94 ± 0.539	-0.338	0.056
CNP	*CNP*	0.902 ± 0.27	0.852 ± 0.338	-0.027	0.803
MBP	*MBP*	1.519 ± 1.213	1.852 ± 1.936	-0.772	0.221
MOG	*MOG*	1.056 ± 0.735	1.219 ± 0.794	-0.421	0.114
PLLP	*PLLP*	0.604 ± 0.166	0.649 ± 0.213	-0.108	0.147
MOBP163	*MOBP*	1.217 ± 0.487	1.178 ± 0.653	-0.01	0.963
MOBP81	*MOBP*	0.856 ± 0.43	0.868 ± 0.496	-0.119	0.514
Appoptosin	*SLC25A38*	0.54 ± 0.216	0.418 ± 0.177	0.157	0.053
BACE1	*BACE1*	1.534 ± 0.655	1.441 ± 0.462	0.098	0.65
APP	*APP*	1.557 ± 0.42	1.285 ± 0.199	0.323	0.018
E1 (Tau)	*MAPT*	2.108 ± 0.632	1.874 ± 0.527	0.266	0.239
β3-tubulin	*TUBB3*	1.383 ± 0.543	1.275 ± 0.621	0.115	0.516
GFAP	*GFAP*	1.075 ± 0.524	1.331 ± 0.911	-0.565	0.048
PS1	*PSEN1*	1.048 ± 0.328	0.938 ± 0.316	0.281	0.02
PS2	*PSEN2*	0.847 ± 0.341	0.835 ± 0.487	0.272	0.068
IBA-1	*AIF1*	1.624 ± 2.551	0.463 ± 0.318	1.076	0.136

Proteome Data of Mayo TCX Samples from Emory/UCLA (84 AD vs. 83 PSP)

Gene Name|UniProt ID	Gene Symbol	log2FC.PSPvsAD	p.PSPvsAD	FDR.PSPvsAD
MBP|P02686	MBP	-0.283166459	0.004054992	**0.013873479**
MBP|H7BYR8	MBP	-0.44204852	0.006880439	**0.021600988**
CNP|P09543	CNP	-0.230290189	0.008508914	**0.025767751**
MOG|C9JTE0	MOG	-0.169774747	0.080079509	0.159454749
PLP1|P60201	PLP1	-0.138230067	0.19583294	0.324337773
BIN1|O00499	BIN1	-0.005061693	0.881080053	0.931413656

Rigor and "External" Reproducibility

ELSEVIER

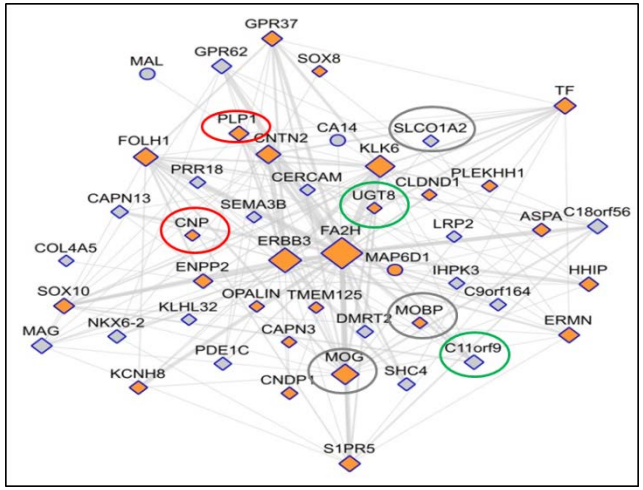
Alzheimer's & Dementia ■ (2017) 1-15

Featured Article

Conserved brain myelination networks are altered in Alzheimer's and other neurodegenerative diseases

(Allen, Ertekin-Taner et al.)

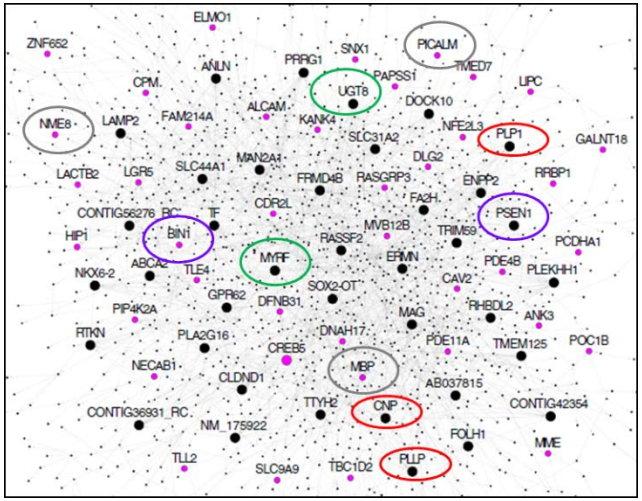
261 AD, 179 PSP, 76 Control (2 cohorts) TCX



Mayo Discovery Cohort

(McKenzie, Zhang et al.)

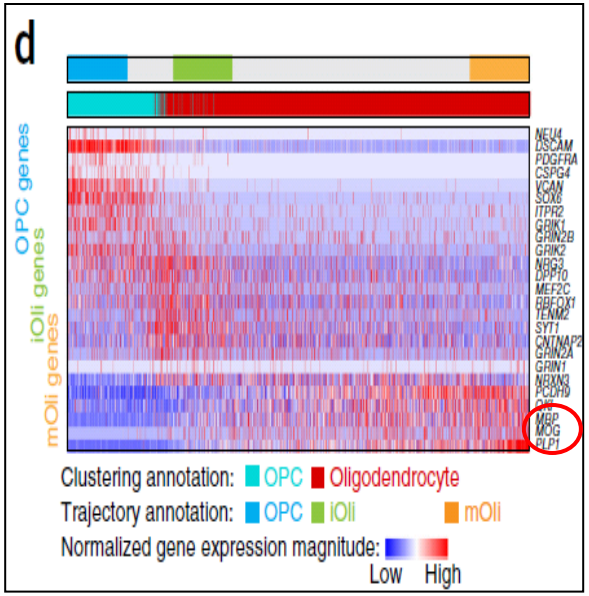
376 AD, 173 non-AD CER, DLPFC, VC



Mount Sinai

(Lake, Zhang et al.)

6 brains CER, FC, VC



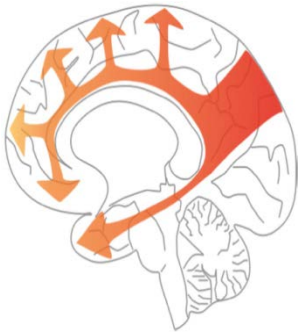
UCSD

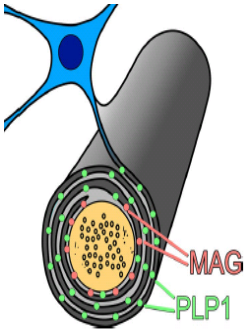
Replication of Network Structure:
Myelination genes: *PLP1, PLLP, CNP, MOBP*
AD risk/related genes: *BACE1, PSEN1, BIN1, UNC5C*
o myelin, o key driver, o AD risk genes (replicable)

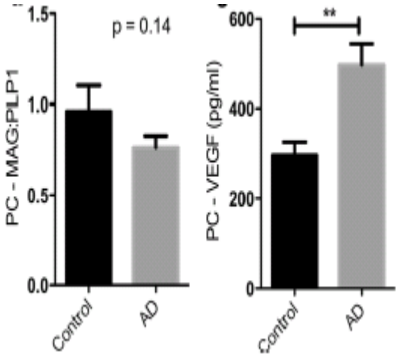
Replication of Myelination genes:
Myelination genes: *PLP1, MOG, MBP*
AD risk/related genes: *MEF2C*
o myelin

Potential Mechanisms of Myelin Dysregulation

- **Hypoperfusion:**



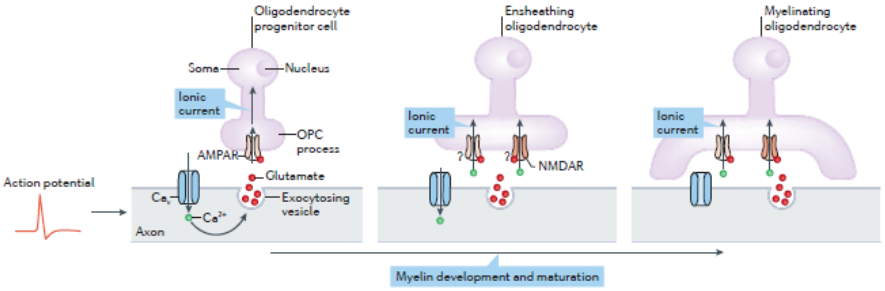




Spread of hypoperfusion in AD

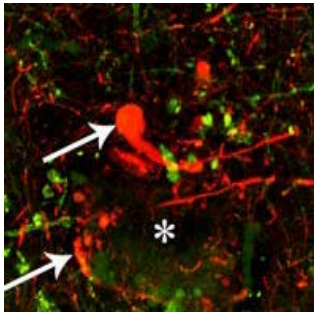
MAG:PLP1 reduced and VEGF increased in AD
(Love&Miners, Acta Neuropath, 2016)

- **Disrupted axo-myelin transmission:**

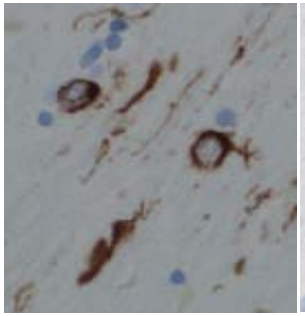


Hypothetical development of axo-myelinic synapse
(Micu et al., Nat Rev NSci, 2018)

- **Protesostasis (Tau, Aß, α-Syn):**

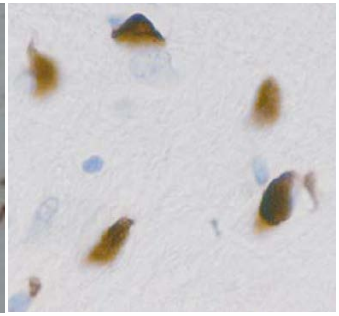


AD, Aß plaque (*), demyelinated axon (arrow)
(Mitew et al., 2010)



PSP, Tau

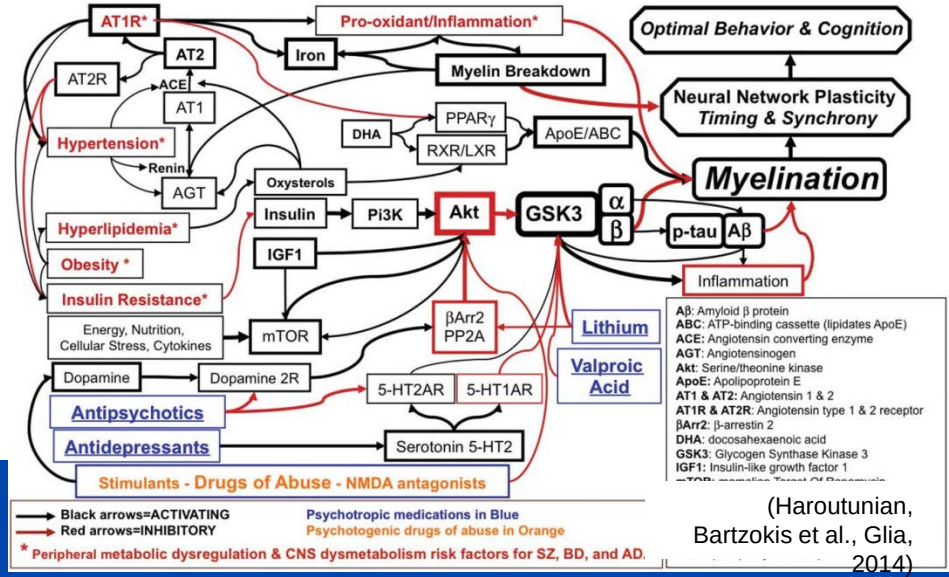
(Dickson et al., 2006)



MSA, α-Syn

(Croisier&Graber, 2006)

- **Neurotransmitters, inflammation:**



(Haroutunian, Bartzokis et al., Glia, 2014)

Novel Target Discovery: Myelin

Therapeutic Hypothesis: Promoting Myelination and Oligodendrocyte Health

We and others have recently implicated oligodendrocyte and myelin dysfunction as an early event in AD (and PSP), perhaps even preceding evidence for overt neuronal dysfunction. If this is the case, promoting oligodendrocyte health and myelination may be a key target for intervention in AD (and other neurodegenerative diseases).

Mayo-UF-ISB Targets: Class Myelin-Oligodendrocyte		ADvsControl_Simple_TCX		ADvsCon_Simple_CER		AD vs Control_Comprehensive_TCX		AD vsCon _Comprehensive_CER	
gene symbol	Predicted therapeutic direction	Modules	GO_Module	Modules	GO_Module	Modules	GO_Module	Modules	GO_Module
MOG	agonism	MM5	axon ensheathment	MM20	axon ensheathment	MM8	NA	MM11	axon ensheathment
MOBP	agonism	MM10	NA	MM48	NA	MM33	NA	MM11	axon ensheathment
SLCO1A2	unknown	MM10	NA	MM48	NA	MM8	NA	MM1	NA
UNC5C	agonism	MM5	axon ensheathment	MM22	cell-cell signaling	MM2	NA	MM24	cell-cell signaling
PLP1	agonism	MM5	axon ensheathment	MM20	axon ensheathment	MM2	NA	MM11	axon ensheathment
PLLP	agonism	MM10	NA	MM48	NA	MM8	NA	MM11	axon ensheathment
BIN1	unknown	MM5	axon ensheathment	MM1	chromosome organizatio	MM2	NA	MM8	NA

Mayo-UF-ISB Targets: Class Myelin-Oligodendrocyte		DEG Comparison Summary		AD vs Control_Simple_TCX_DEG		AD vs Control_Simple_CER_DEG		AD vs Control_Comprehensive_TCX_DEG		AD vs Control_Comprehensive_ CER_DEG	
gene symbol	Predicted therapeutic direction	Consistency of TCX and CER	Consistency of Simple vs. Comprehensive Models	FDR	Direction	FDR	Direction	FDR	Direction	FDR	Direction
MOG	agonism	No	No	8.68E-01	HighInAD	5.95E-01	LowInAD	8.16E-01	LowInAD	7.33E-01	LowInAD
MOBP	agonism	Yes	Yes	1.21E-01	LowInAD	**1.50E-02**	**LowInAD**	7.77E-02	LowInAD	**3.17E-02**	**LowInAD**
SLCO1A2	unknown	Yes	Yes	7.52E-01	LowInAD	9.11E-01	LowInAD	1.80E-01	LowInAD	9.67E-01	LowInAD
UNC5C	agonism	No	Yes	6.95E-01	HighInAD	1.41E-01	LowInAD	7.78E-01	HighInAD	5.53E-01	LowInAD
PLP1	agonism	No	Yes	3.25E-01	HighInAD	9.28E-01	LowInAD	8.17E-01	HighInAD	8.68E-01	HighInAD
PLLP	agonism	Yes	Yes	7.35E-01	LowInAD	6.29E-02	LowInAD	5.76E-01	LowInAD	9.43E-02	LowInAD
BIN1	unknown	Yes	Yes	8.25E-01	HighInAD	3.58E-01	HighInAD	9.12E-01	HighInAD	9.30E-01	LowInAD

Conclusions and Implications

- **Myelination networks are down in both AD and PSP, but more so in PSP.**
  - Convergent pathway for multiple neurodegenerative diseases.
  - Tau-related (especially 4R), disrupted neuron-glia interaction, other?
  - Role of aging and high metabolic demand of maintaining myelin.
- **Myelination networks are reproducible, validated and their alterations are unlikely to be due to cell population changes.**
  - TCX is a relatively unaffected region in PSP.
  - Similar findings even after adjusting for cell populations.
  - Internal, external replications, including single cell type data.
- **Myelination networks harbor AD and PSP risk genes.**
  - Mechanistic implications for these genes and their variants.
- **Implications for (Combination) Therapy.**
  - Myelin repair/remyelination.
  - Maintenance of microglial, astrocyte function (myelin debris removal)
  - APOE/lipid metabolism/cerebrovascular health

Comparative Multi-omics and Divergent Neurodegenerative Disease Mechanisms:

Innate Immunity

nature genetics

Rare coding variants in *PLCG2*, *ABI3*, and *TREM2* implicate microglial-mediated innate immunity in Alzheimer's disease

We identified rare coding variants associated with Alzheimer's disease in a three-stage case–control study of 85,133 subjects. In stage 1, we genotyped 34,174 samples using a whole-exome microarray. In stage 2, we tested associated variants ($P < 1 \times 10^{-4}$) in 35,962 independent samples using *de novo* genotyping and imputed genotypes. In stage 3, we used an additional 14,997 samples to test the most significant stage 2 associations ($P < 5 \times 10^{-8}$) using imputed genotypes. We observed three new genome-wide significant nonsynonymous variants associated with Alzheimer's disease: a protective variant in *PLCG2* (rs72824905: p.Pro522Arg, $P = 5.38 \times 10^{-10}$, odds ratio (OR) = 0.68, minor allele frequency $(MAF)_{cases} = 0.0059$, $MAF_{controls} = 0.0093$), a risk variant in *ABI3* (rs616338: p.Ser209Phe, $P = 4.56 \times 10^{-10}$, OR = 1.43, $MAF_{cases} = 0.011$, $MAF_{controls} = 0.008$), and a new genome-wide significant variant in *TREM2* (rs143332484: p.Arg62His, $P = 1.55 \times 10^{-14}$, OR = 1.67, $MAF_{cases} = 0.0143$, $MAF_{controls} = 0.0089$), a known susceptibility gene for Alzheimer's disease. These protein-altering changes are in genes highly expressed in microglia and highlight an immune-related protein–protein interaction network enriched for previously identified risk genes in Alzheimer's disease. These genetic findings provide additional evidence that the microglia-mediated innate immune response contributes directly to the development of Alzheimer's disease.

controls using the Illumina HumanExome microarray. Data from multiple consortia were combined in a single-variant meta-analysis (Online Methods) assuming an additive model. In total, 241,551 variants passed quality control (**Supplementary Table 3**). Of these, 203,902 were polymorphic, 26,947 were common (MAF ≥ 5%), and 176,955 were low frequency or rare (MAF < 5%). We analyzed common variants using a logistic regression model in each sample cohort and combined data using METAL[30]. Rare and low-frequency variants were analyzed using the score test and data were combined with SeqMeta[31] (**Supplementary Fig. 2**).

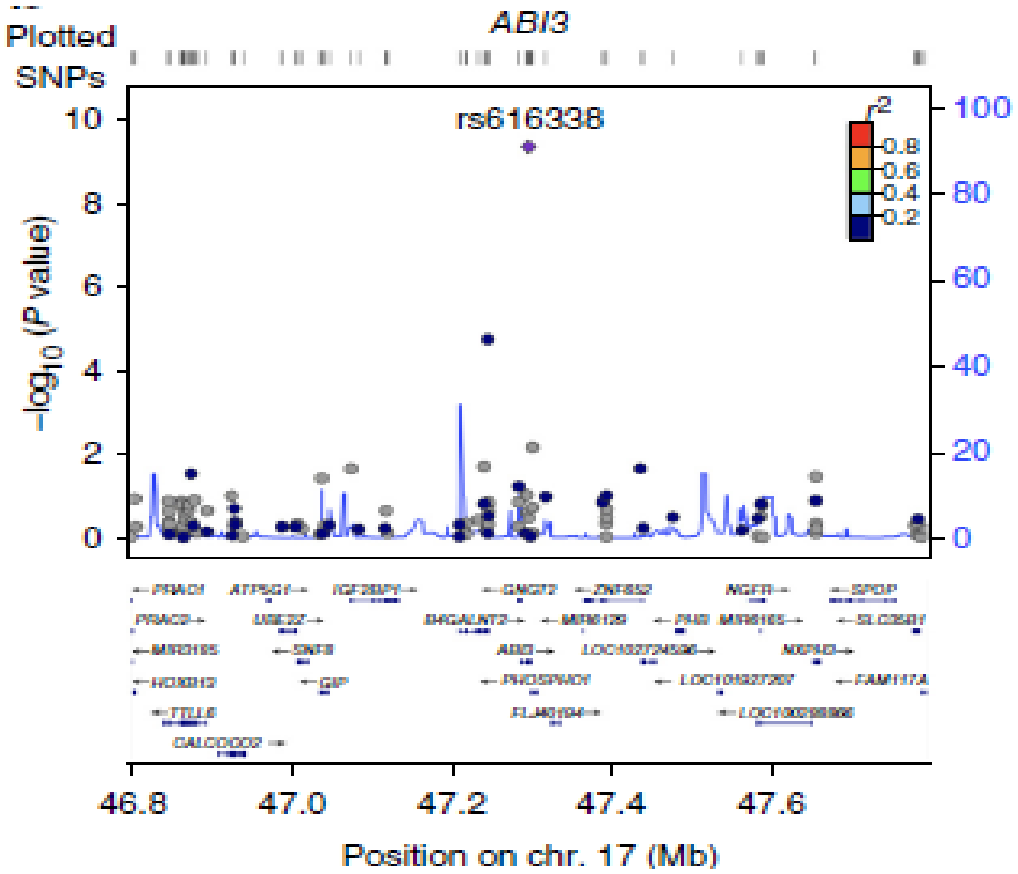
We reviewed cluster plots for variants showing association ($P < 1 \times 10^{-4}$) and identified 43 candidate variants (**Supplementary Table 4**), excluding known risk loci (**Supplementary Table 5**). In stage 2, we tested these for association in 14,041 LOAD cases and 21,921 controls, using genotypes derived from *de novo* genotyping and imputation (Online Methods). We carried forward single-nucleotide variants (SNVs) with genome-wide significant associations and consistent directions of effect to stage 3 where genotypes for 6,652 independent cases and 8,345 controls were imputed using the Haplotype Reference Consortium resource[32,33] (Online Methods and **Supplementary Table 6**).

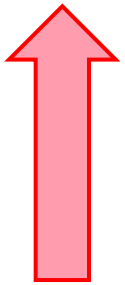
We identified four rare coding variants with genome-wide significant association signals with LOAD ($P < 5 \times 10^{-8}$) (**Table 2** and **Supplementary Tables 7 and 8**). The first is a missense variant p.Pro522Arg ($P = 5.38$

Table 2 Summary of stages 1, 2 and 3 and combined meta-analysis results for SNVs at $P < 5 \times 10^{-8}$

SNV	rs75932628	rs143332484	rs72824905	rs616338
Chr.	6	6	16	17
Position (bp)	41,129,252	41,129,207	81,942,028	47,297,297
Protein variation	Arg47His	Arg62His	Pro522Arg	Ser209Phe
Gene	*TREM2*	*TREM2*	*PLCG2*	*ABI3*
Effect allele	T	T	G	T
Stage 1				
P	3.02×10^{-12}	3.48×10^{-9}	1.19×10^{-5}	2.16×10^{-5}
OR	2.46	1.58	0.65	1.42
MAF_{cases}	0.003	0.015	0.006	0.013
$MAF_{controls}$	0.001	0.010	0.011	0.010
N	30,018	33,786	33,786	33,786
Stage 2				
P	4.38×10^{-8}	3.66×10^{-7}	1.35×10^{-4}	8.37×10^{-5}
OR	2.37	3.97	0.70	1.41
MAF_{cases}	0.004	0.014	0.006	0.010
$MAF_{controls}$	0.002	0.006	0.008	0.008
N	35,831	3,968	35,831	35,831
Stage 3				
P	1.23×10^{-6}	2.45×10^{-3}	2.48×10^{-2}	1.75×10^{-2}
OR	2.58	1.55	0.69	1.58
MAF_{cases}	0.006	0.012	0.006	0.010
$MAF_{controls}$	0.003	0.008	0.007	0.008
N	14,884	15,288	15,288	14,876
Stage 1–3 meta-analysis				
P	5.38×10^{-24}	1.55×10^{-14}	5.38×10^{-10}	4.56×10^{-10}
OR	2.46	1.67	0.68	1.43
MAF_{cases}	0.004	0.014	0.006	0.011
$MAF_{controls}$	0.002	0.009	0.009	0.008
N	80,733	53,042	84,905	84,493

Abelson Interactor Protein 3 (*ABI3*) Ser209Phe, rs616338





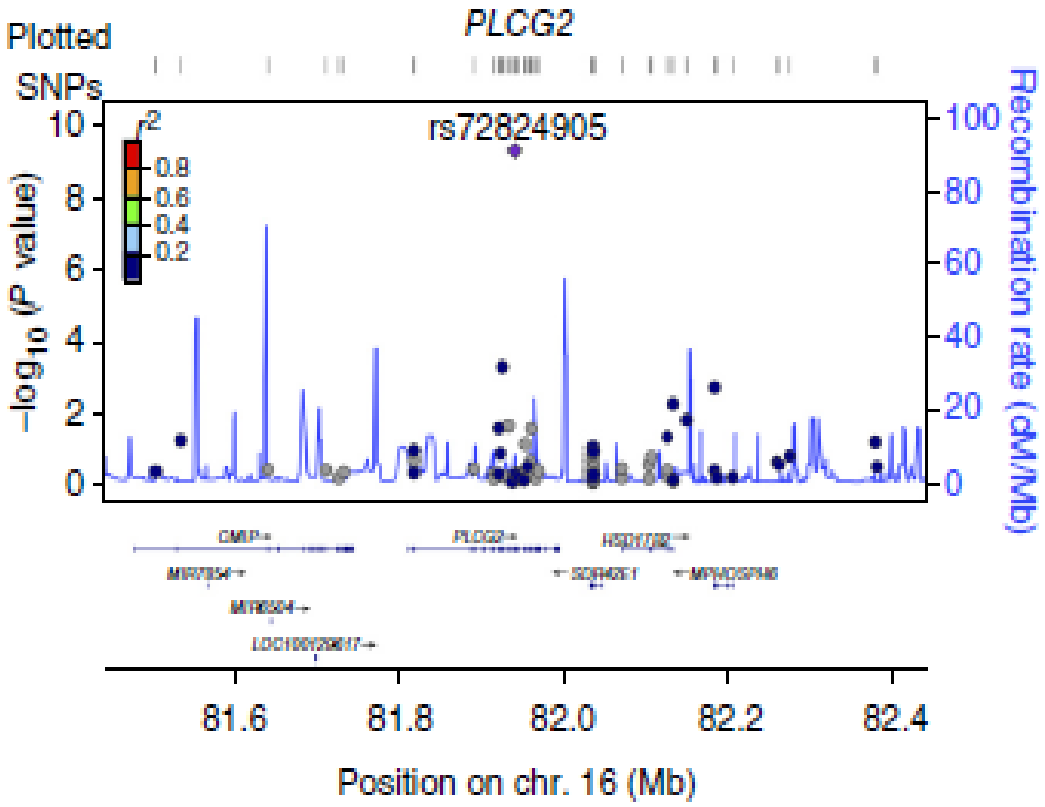
Risk of Alzheimer's Disease

Odds Ratio (OR)	**1.43**
P value	**4.56×10^{-10}**
Minor Allele Frequency Cases	**0.011**
Minor Allele Frequency Controls	**0.008**

MAYO CLINIC

Sims *et al*., 2017

Phospholipase C γ2 (*PLCG2*) Pro522Arg, rs72824905



Risk of Alzheimer's Disease

Odds Ratio (OR)	**0.68**
P value	**5.38×10^{-10}**
Minor Allele Frequency Cases	**0.0059**
Minor Allele Frequency Controls	**0.0093**

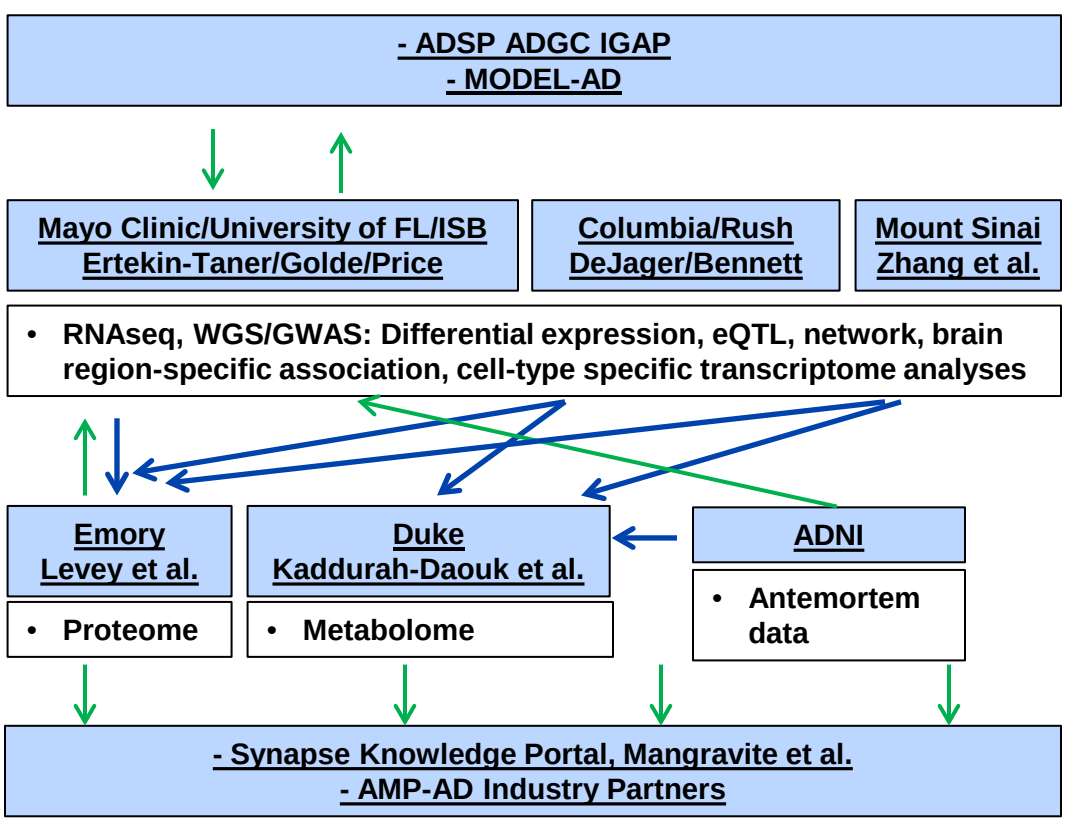
MAYO CLINIC

Sims *et al*., 2017

Conclusions and Implications

- **Innate immunity/microglial networks are up only in AD, but not in PSP.**
  - Divergent pathway between AD vs. PSP (primary tauopathy).
  - AD (Aβ) specificity?
- **Innate immunity networks are reproducible and validated though their changes are likely due to microgliosis in AD pathology-affected regions.**
  - Observed only in AD vs. control, in TCX and simple model.
  - Findings disappear after adjusting for cell populations.
- **Innate immunity networks harbor AD risk genes.**
  - Mechanistic implications for these genes and their variants.
- **Implications for Therapy.**
  - Innate immunity may be a viable AD-specific target.
  - Opposite direction of risk associations between AD and PSP for some innate immunity AD risk genes may be multifactorial and should raise caution about targeting innate immunity in non-AD degenerative diseases.

AMP-AD Interactive Collaborations



- Annotate disease genes and variants.
- Prioritize disease models by variants and mechanism.

Drug Targets

- Replication in independent cohorts
- Validation in model systems
- Identification of timing of alterations with respect to disease progression
- Discovery of mechanisms of alteration
- Identification of beneficial vs. detrimental direction of effect.

Open Science

- Wide sharing of data.
- Data visualization tools
- Education of research community

- Simplified schematic depiction of the ongoing and planned collaborations with AMP-AD and other partners.
- Blue arrows: Shared samples. Green arrows: Shared data. Red arrows: Expected outcomes.
- Data generated by the teams are shown in white boxes below the relevant teams.
- This figure highlights the specific data types shared with and by our team and is not a full inventory of all data by all groups. Our team also widely shares rAAV tools and mouse brain data with all teams (not shown).

