Integrative translational discovery of vascular risk factors in aging and dementia

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Molecular Mechanisms of the Vascular Etiology of AD (M^{2}OVE-AD)

NIA-AA Symposium: Enabling precision medicine for Alzheimer’s disease through open science
July 19-July 20, 2018; Chicago

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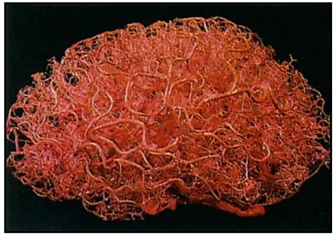
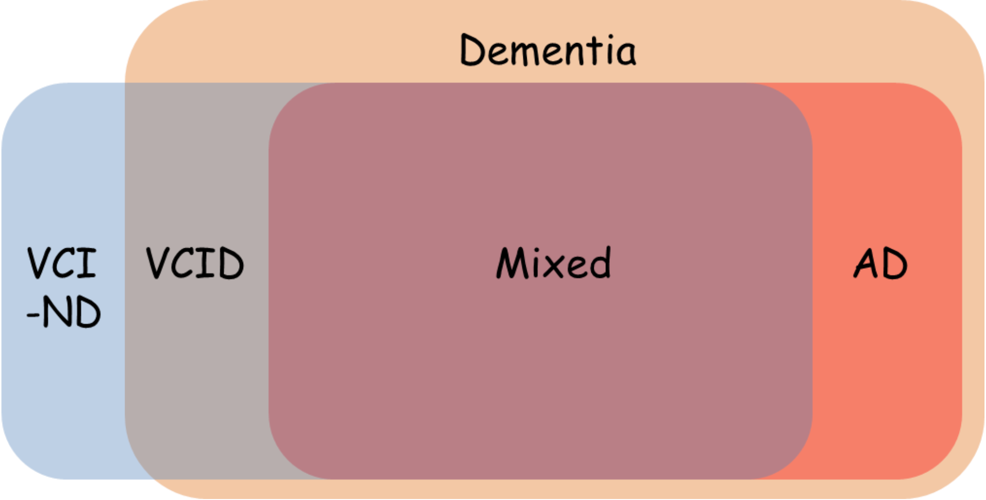
Cerebrovascular contribution to dementia





- AD is the most common cause of dementia accounting for 60-80% of dementia cases.
- In individuals with Alzheimer’s dementia, ~90% of cases have cerebrovascular lesions including cerebral amyloid angiopathy (CAA).

How vascular pathology and risk factors converge with AD?

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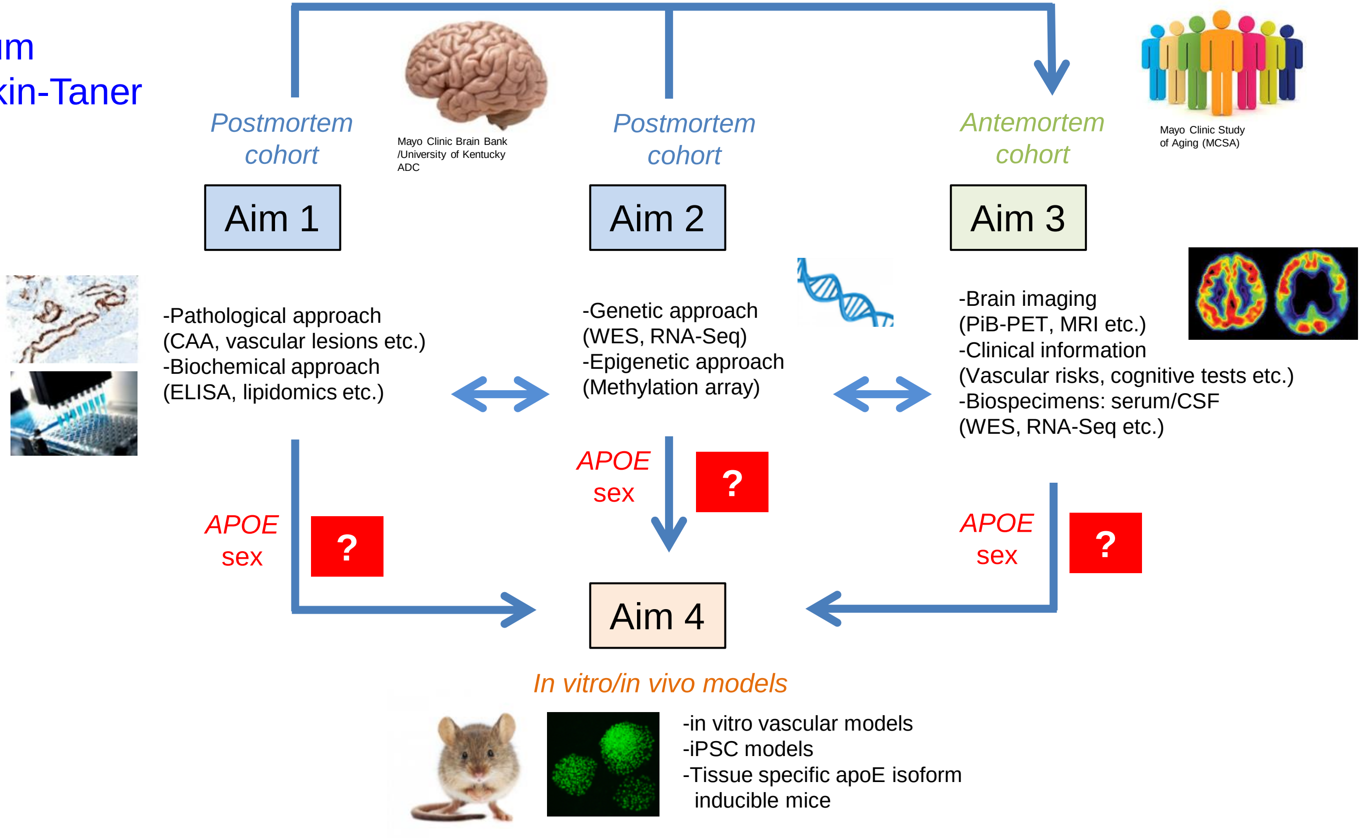
Multidisciplinary approaches to define vascular risk factors in aging, AD and dementia

M^{2}OVE-AD Consortium
Multi-PI: Bu and Ertekin-Taner
RF1AG051504
RF1AG051504-01S2



Postmortem cohort

Mayo Clinic Brain Bank /University of Kentucky ADC

Aim 1

-Pathological approach
(CAA, vascular lesions etc.)
-Biochemical approach
(ELISA, lipidomics etc.)

APOE
sex
?

Postmortem cohort

Aim 2

-Genetic approach
(WES, RNA-Seq)
-Epigenetic approach
(Methylation array)

APOE
sex
?

Antemortem cohort

Mayo Clinic Study of Aging (MCSA)

Aim 3

-Brain imaging
(PiB-PET, MRI etc.)
-Clinical information
(Vascular risks, cognitive tests etc.)
-Biospecimens: serum/CSF
(WES, RNA-Seq etc.)

APOE
sex
?

Aim 4

In vitro/in vivo models

-in vitro vascular models
-iPSC models
-Tissue specific apoE isoform inducible mice

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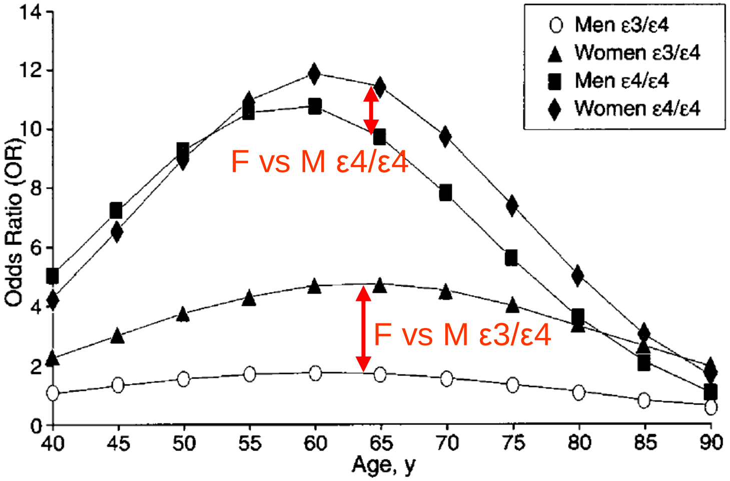
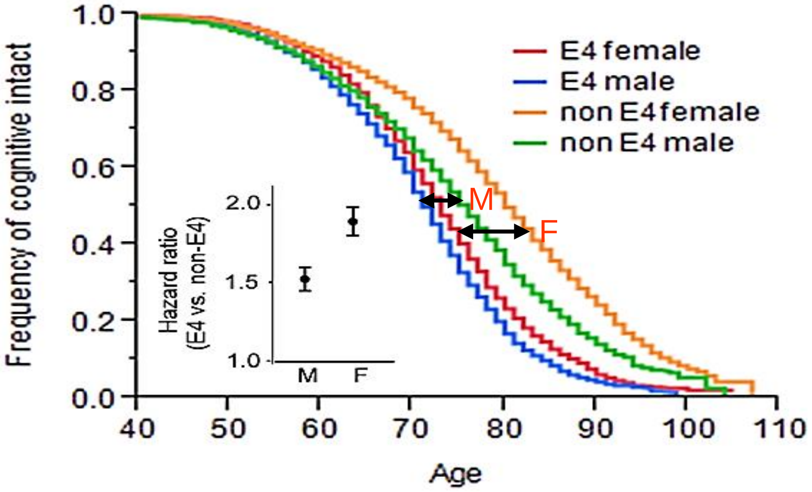
APOE ε4 allele has a greater effect on AD risk/cognitive decline in females than males – sex-dependent effects





- Peak effect ages: 55-70
- Greater ORs in females than in males

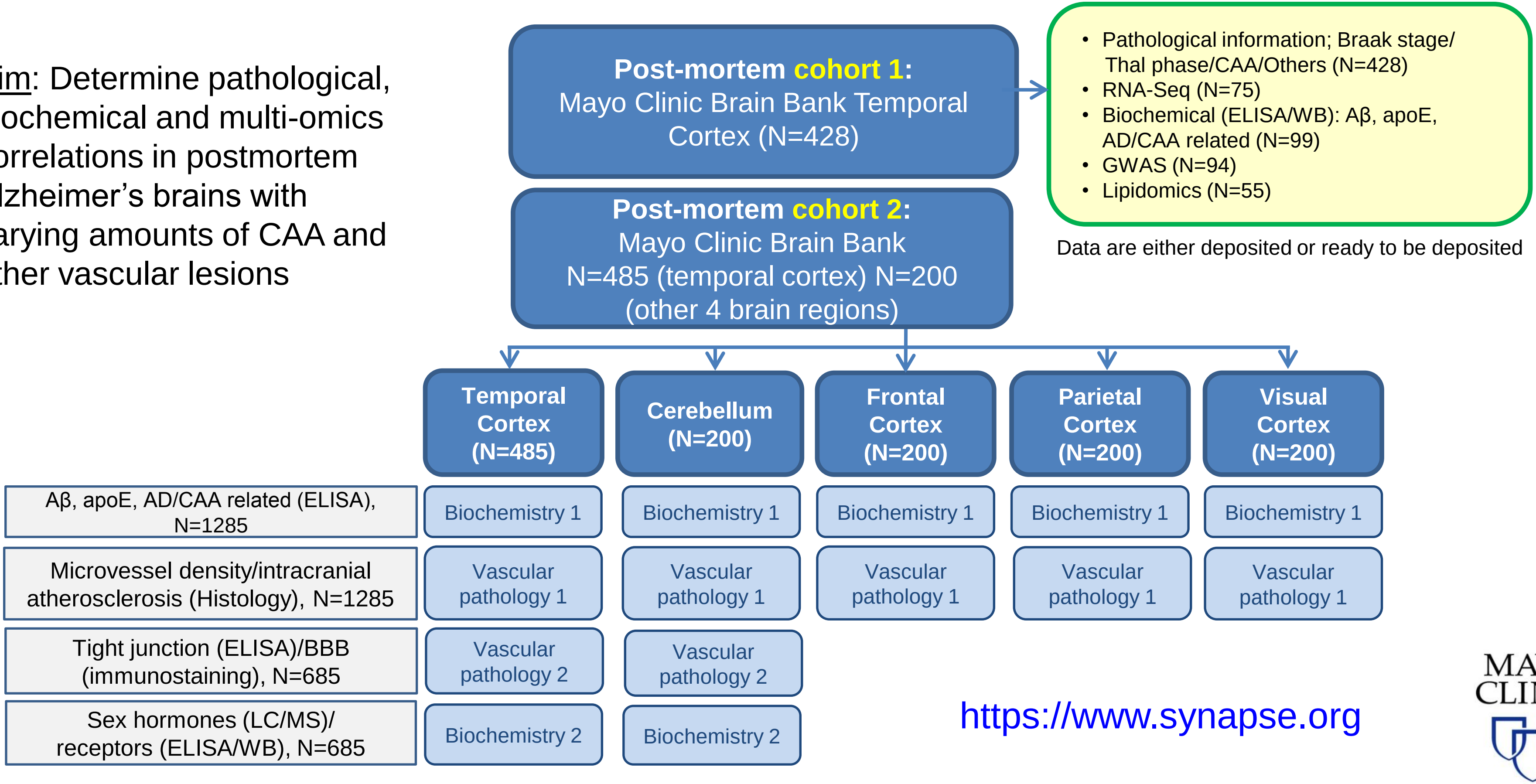
Farrer et al., JAMA, 1997

Cross sectional analysis of 21,531 subjects from National Alzheimer's Coordinating Center (NACC)

Shinohara et al., Ann Neurol, 2016

Impact of *APOE* and sex on vascular pathologies: Pathological, biochemical, and multi-omics studies using postmortem brains

Aim: Determine pathological, biochemical and multi-omics correlations in postmortem Alzheimer's brains with varying amounts of CAA and other vascular lesions



Post-mortem cohort 1:
Mayo Clinic Brain Bank Temporal Cortex (N=428)

- Pathological information; Braak stage/ Thal phase/CAA/Others (N=428)
- RNA-Seq (N=75)
- Biochemical (ELISA/WB): Aβ, apoE, AD/CAA related (N=99)
- GWAS (N=94)
- Lipidomics (N=55)

Data are either deposited or ready to be deposited

Post-mortem cohort 2:
Mayo Clinic Brain Bank
N=485 (temporal cortex) N=200 (other 4 brain regions)

	Temporal Cortex (N=485)	**Cerebellum (N=200)**	**Frontal Cortex (N=200)**	**Parietal Cortex (N=200)**	**Visual Cortex (N=200)**
Aβ, apoE, AD/CAA related (ELISA), N=1285	Biochemistry 1	Biochemistry 1	Biochemistry 1	Biochemistry 1	Biochemistry 1
Microvessel density/intracranial atherosclerosis (Histology), N=1285	Vascular pathology 1	Vascular pathology 1	Vascular pathology 1	Vascular pathology 1	Vascular pathology 1
Tight junction (ELISA)/BBB (immunostaining), N=685	Vascular pathology 2	Vascular pathology 2			
Sex hormones (LC/MS)/ receptors (ELISA/WB), N=685	Biochemistry 2	Biochemistry 2			

https://www.synapse.org

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Subject Characteristics - Postmortem Human Brain Cohort 1

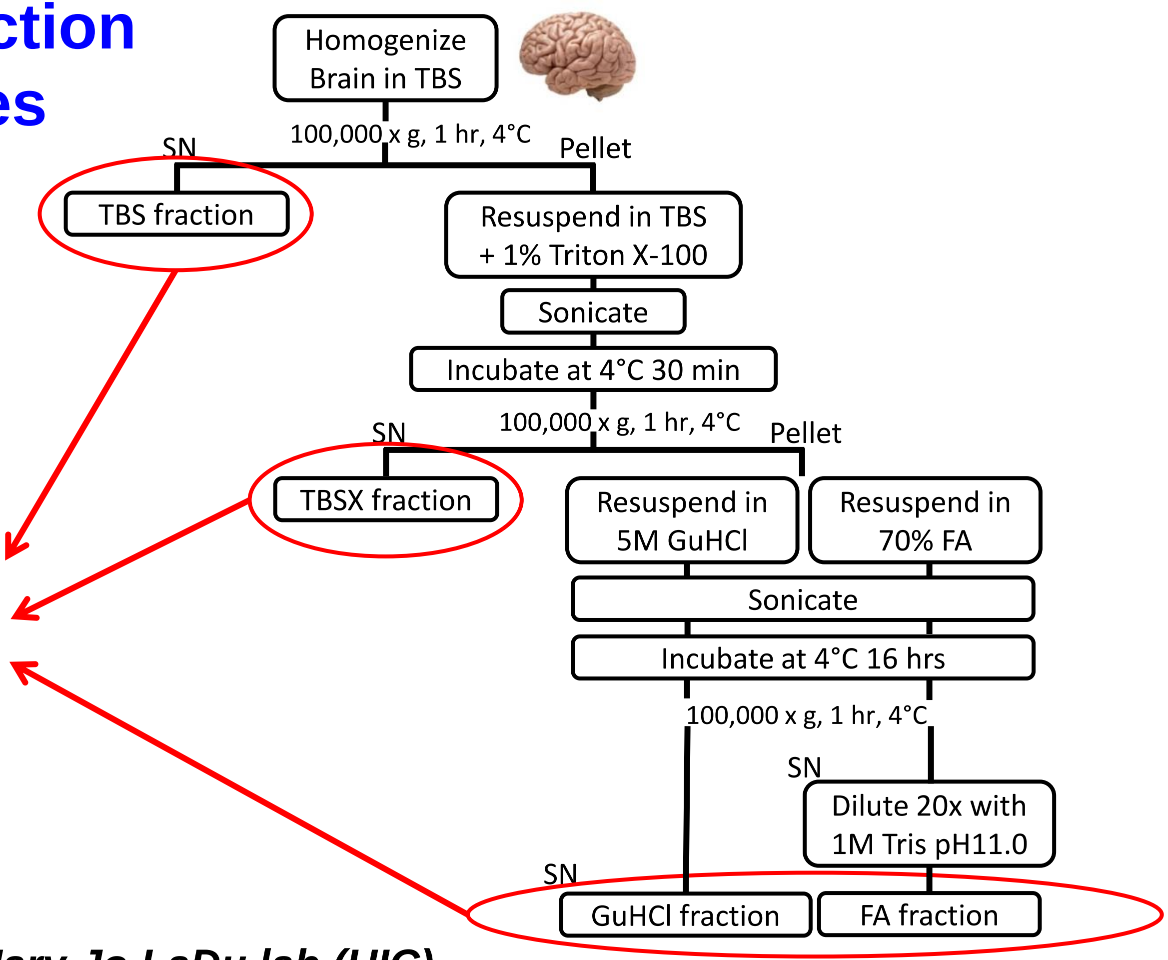
Pathologically-confirmed AD cases

	APOE4 non-carrier		*APOE4* carrier	
	Female (N=103)	Male (N=68)	Female (N=143)	Male (N=114)
Age	81 (75, 88)	80 (73, 85)	83 (78, 88)	79 (73, 83)
Braak Stage				
<5	12 (12)	8 (12)	11 (8)	10 (9)
5-5.5	39 (38)	27 (40)	39 (27)	45 (39)
6	52 (50)	33 (49)	93 (65)	59 (52)
Thal Stage				
Missing	1	0	2	2
<4	11 (11)	6 (9)	8 (6)	11 (10)
4	8 (8)	7 (10)	11 (8)	10 (9)
5	83 (81)	55 (81)	122 (87)	91 (81)

Median and interquartile range (IQR) shown for age; number (%) shown for Braak and Thal stages.

ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	total
n=12	n=9	n=159	n=188	n=60	n=428

Three-step extraction of brain tissues



Homogenize Brain in TBS

100,000 x g, 1 hr, 4°C

SN

TBS fraction

Pellet

Resuspend in TBS + 1% Triton X-100

Sonicate

Incubate at 4°C 30 min

100,000 x g, 1 hr, 4°C

SN

TBSX fraction

Pellet

Resuspend in 5M GuHCl

Resuspend in 70% FA

Sonicate

Incubate at 4°C 16 hrs

100,000 x g, 1 hr, 4°C

SN

Dilute 20x with 1M Tris pH11.0

SN

GuHCl fraction

FA fraction

Biochemical analysis

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Co-developed with Mary Jo LaDu lab (UIC)

Associations of sex and *APOE4* with CAA score

		Sex and *APOE4* in model [c]		Additionally adjusting for age, Braak stage and Thal stage [c]	
Covariate	Subgroup	Effect (95% CI)	p-value [d]	Effect (95% CI)	p-value [d]
Male	All [a] (n=428)	0.25 (0.10, 0.40)	**<.001**	0.27 (0.12, 0.42)	**<.001**
	APOE4- [b] (n=171)	0.27 (0.03, 0.51)	**0.027**	0.29 (0.05, 0.53)	**0.017**
	APOE4+ [b] (n=257)	0.24 (0.05, 0.43)	**0.014**	0.26 (0.06, 0.46)	**0.010**
APOE4+	All [a] (n=428)	0.23 (0.08, 0.38)	**0.003**	0.22 (0.06, 0.37)	**0.005**
	Females [b] (n=246)	0.24 (0.04, 0.44)	**0.017**	0.23 (0.03, 0.43)	**0.024**
	Males [b] (n=182)	0.21 (-0.02, 0.45)	0.074	0.20 (-0.03, 0.43)	0.095
***APOE4* allelic dose**	All [a]	0.30 (0.20, 0.41)	**<.001**	0.30 (0.19, 0.40)	**<.001**
	Females [b]	0.30 (0.16, 0.44)	**<.001**	0.29 (0.15, 0.43)	**<.001**
	Males [b]	0.30 (0.15, 0.46)	**<.001**	0.31 (0.15, 0.46)	**<.001**

[a] Results from models with no interaction terms. [b] Results from models with interaction of *APOE4* and sex. [c] Age is included as a continuous variable, Braak stage as dichotomous (6 vs. <6), and Thal as dichotomous (5 vs. <5). [d] Wald p-value.

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Shinohara et al., Acta Neuropathol. 2016

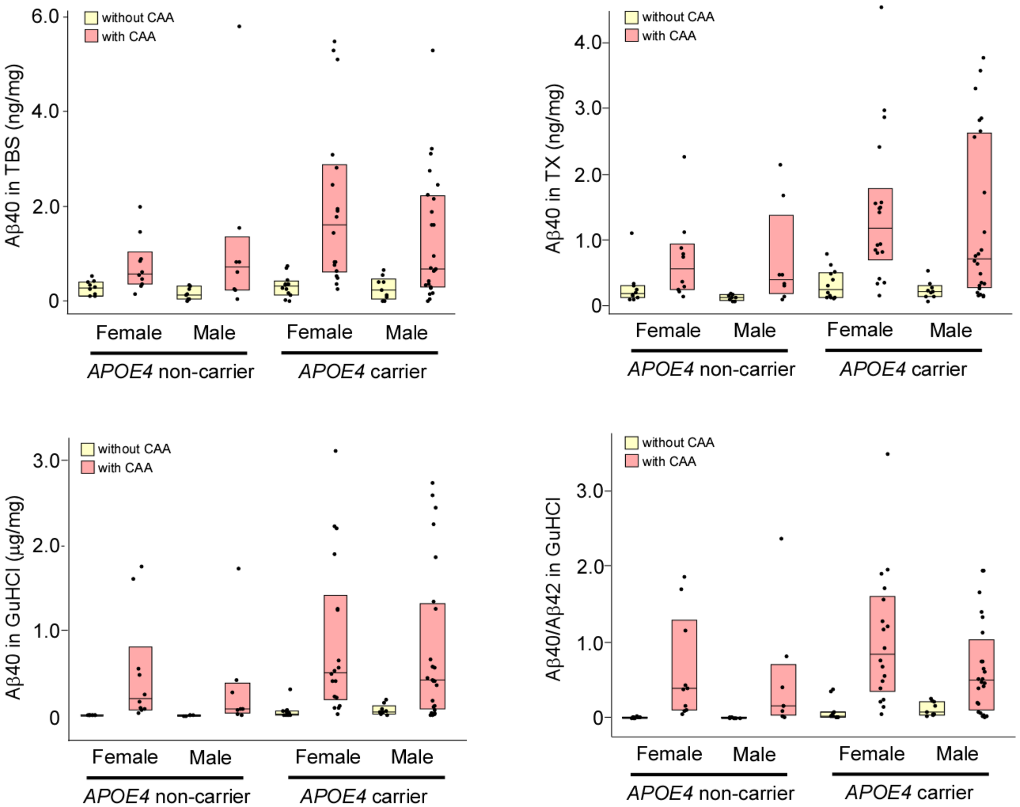
Associations of CAA, sex, and *APOE4* with Aβ and apoE in temporal cortex

	TBS		TX		GuHCl	
	Relative Level [a] (95% CI)	p-value [b]	Relative Level [a] (95% CI)	p-value [b]	Relative Level [a] (95% CI)	p-value [b]
Aβ40						
CAA+	3.50 (2.45, 5.00)	**<.001**	3.64 (2.37, 5.59)	**<.001**	17.55 (10.17, 30.29)	**<.001**
Sex; Male	0.71 (0.50, 1.00)	0.053	0.77 (0.51, 1.16)	0.213	0.68 (0.40, 1.17)	0.161
APOE4+	1.71 (1.20, 2.46)	**0.003**	1.61 (1.06, 2.47)	**0.027**	3.99 (2.30, 6.92)	**<.001**
Aβ42						
CAA+	0.88 (0.67, 1.17)	0.377	1.74 (0.99, 3.07)	0.053	1.22 (0.98, 1.51)	0.077
Sex; Male	1.10 (0.83, 1.45)	0.513	0.99 (0.57, 1.73)	0.978	1.15 (0.93, 1.42)	0.208
APOE4+	0.92 (0.69, 1.22)	0.575	1.26 (0.72, 2.22)	0.423	1.10 (0.88, 1.37)	0.387
Aβ40/Aβ42						
CAA+	3.97 (2.67, 5.92)	**<.001**	2.30 (1.43, 3.69)	**<.001**	14.43 (8.74, 23.83)	**<.001**
Sex; Male	0.64 (0.43, 0.96)	**0.029**	0.91 (0.57, 1.44)	0.676	0.59 (0.36, 0.97)	**0.038**
APOE4+	1.86 (1.24, 2.78)	**0.003**	1.13 (0.70, 1.81)	0.624	3.62 (2.18, 6.01)	**<.001**
ApoE						
CAA+	1.08 (0.94, 1.25)	0.275	1.07 (0.92, 1.24)	0.409	1.69 (1.15, 2.48)	**0.007**
Sex; Male	1.09 (0.95, 1.26)	0.231	1.15 (0.99, 1.34)	0.068	0.74 (0.51, 1.08)	0.119
APOE4+	0.80 (0.69, 0.93)	**0.003**	0.96 (0.83, 1.13)	0.649	1.69 (1.15, 2.49)	**0.008**

[a] Analyses use logarithm of biochemical measures as response variable in linear regression analyses including variables CAA group (severe versus none), sex (male vs. female) and *APOE4* (presence vs. absence) and also adjusting for age; estimated effects are exponentiated to provide effect expressed as a relative level or fold change. [b] Wald p-value.

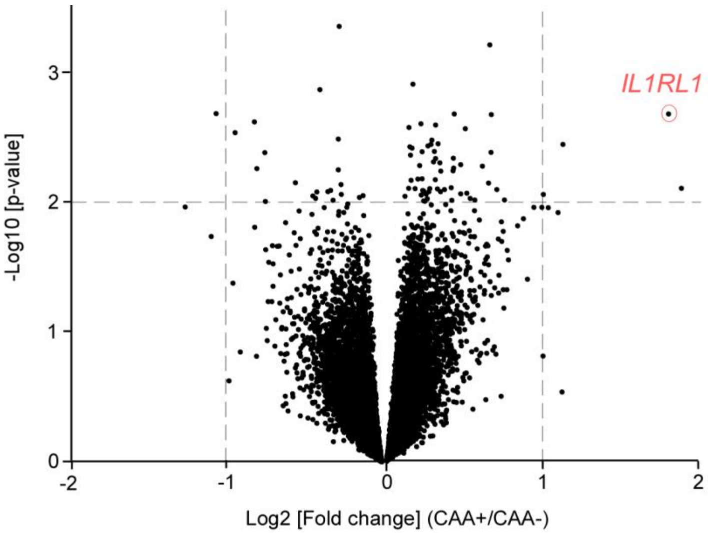
Shinohara et al., Acta Neuropathol. 2016

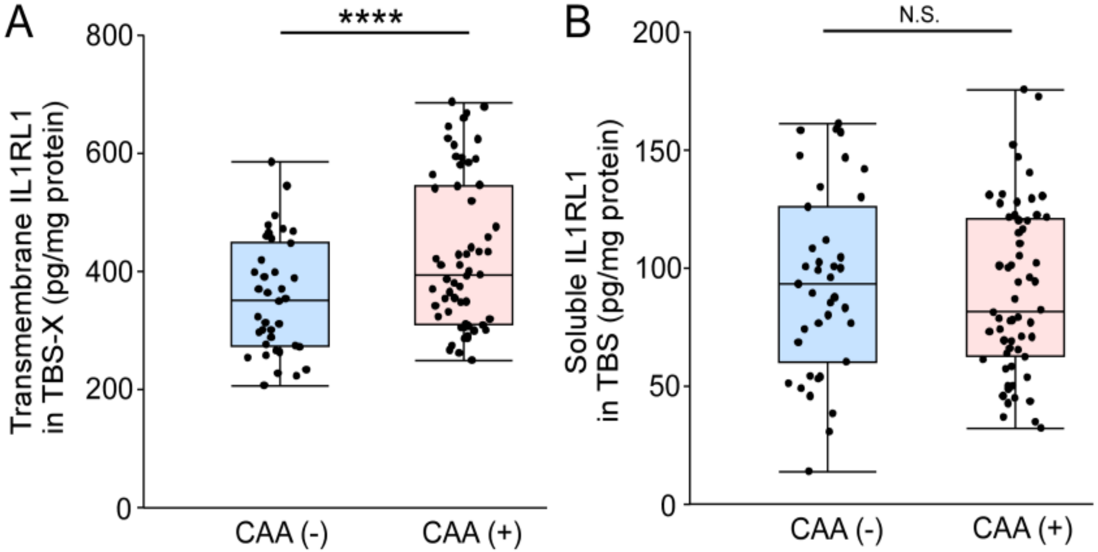
CAA-, sex- and *APOE4*-dependent effects on Aβ40 in the temporal cortex



Shinohara et al., Acta Neuropathol. 2016

IL1RL1 expression is upregulated in AD cases with CAA: transcriptomics studies

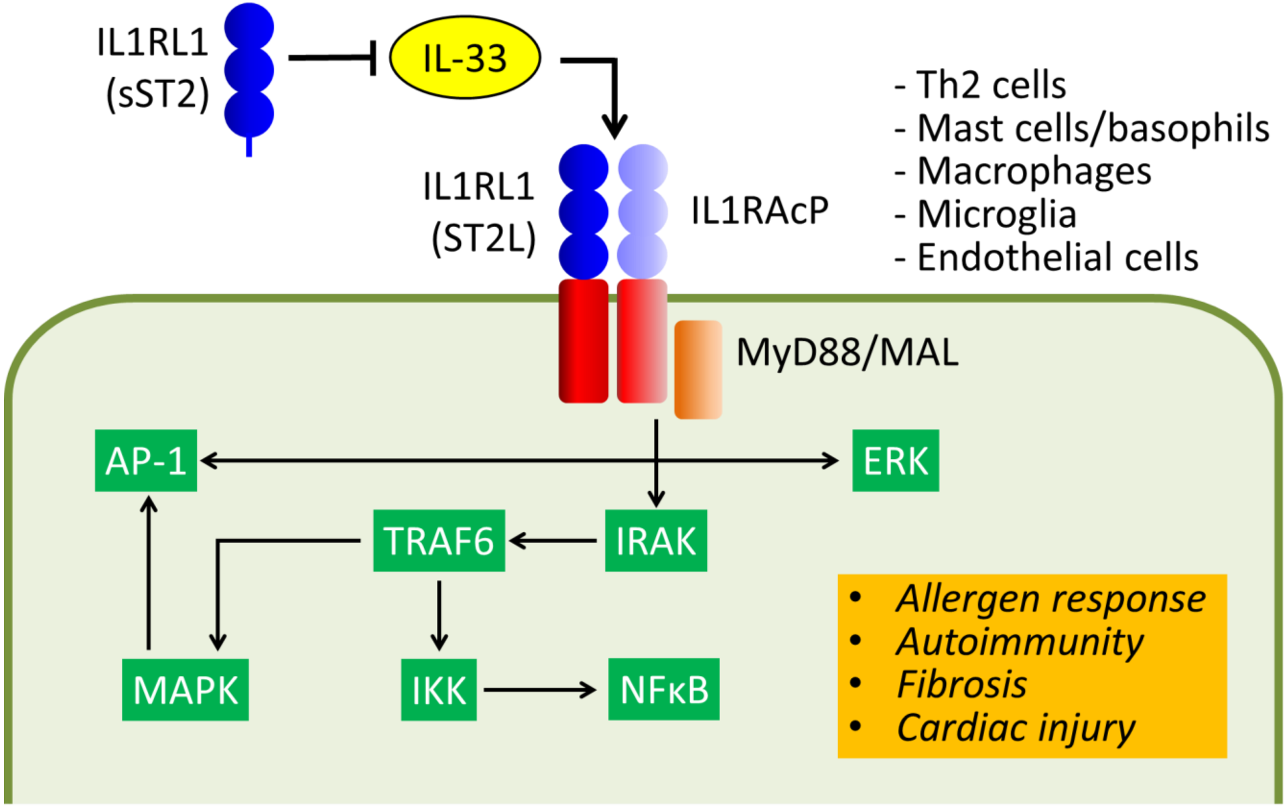




Transcripts in the temporal cortex from AD cases with severe CAA (n=43) or without CAA (n=32) were analyzed by RNA-seq.

The Mayo Clinic AD-CAA RNA-Seq (MC-CAA) study
https://www.synapse.org/#!Synapse:syn9779506

IL1RL1/IL-33 pathway



- Th2 cells
- Mast cells/basophils
- Macrophages
- Microglia
- Endothelial cells

- *Allergen response*
- *Autoimmunity*
- *Fibrosis*
- *Cardiac injury*

R. Kakkar, R.T. Lee, Nat Rev Drug Discov, 7 (2008), pp. 827-840

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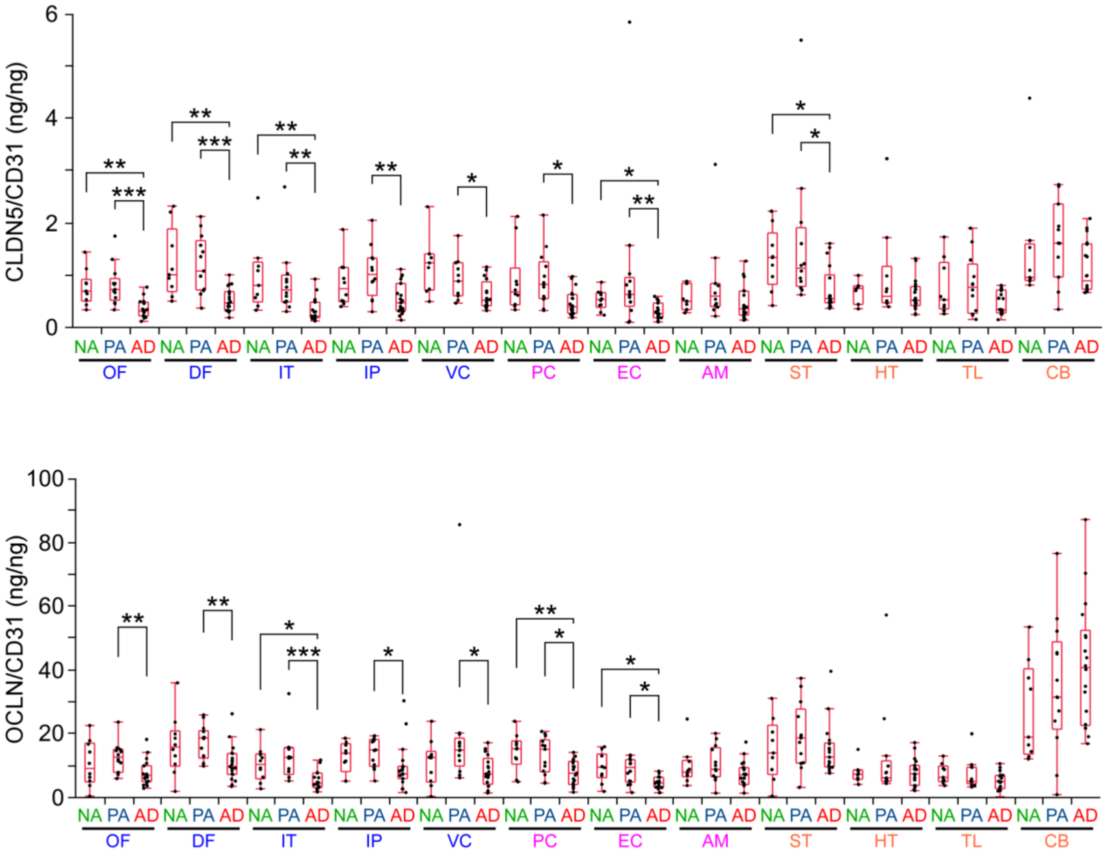
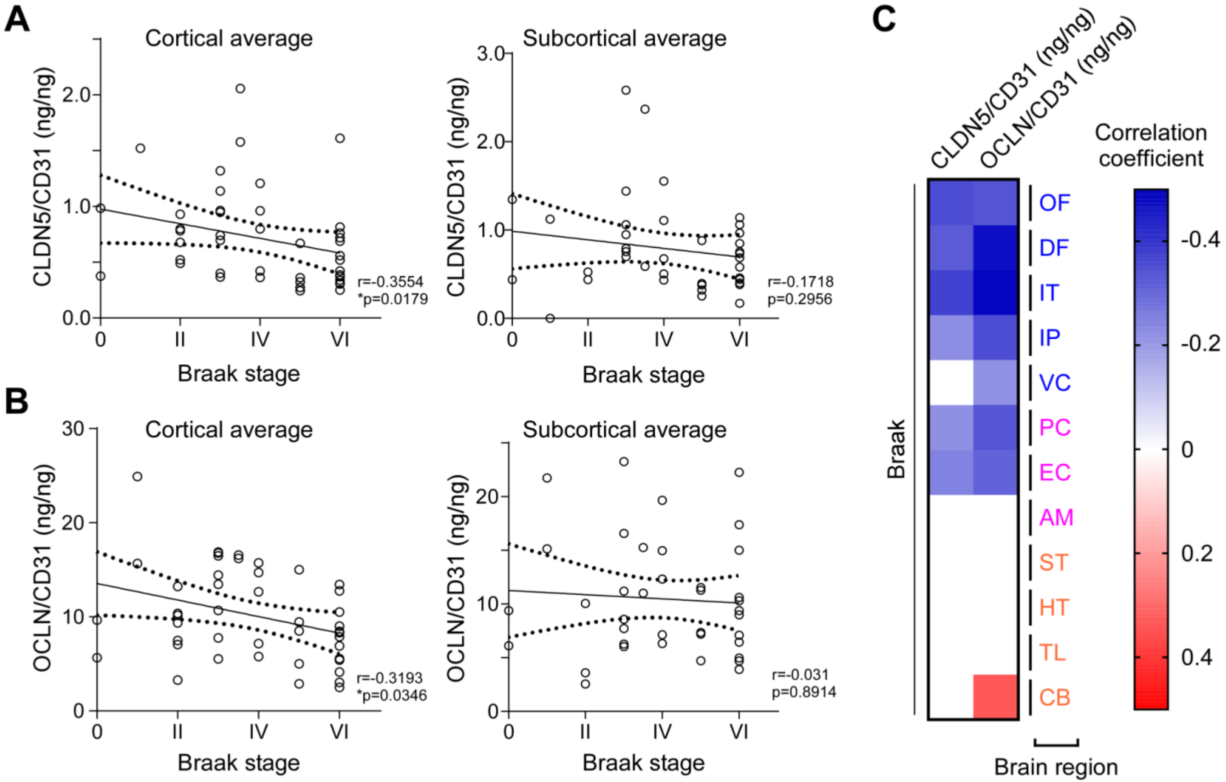
Tight junction proteins are selectively decreased in cortical regions during AD progression



NA: normal aging
PA: pathological aging
AD: Alzheimer's disease

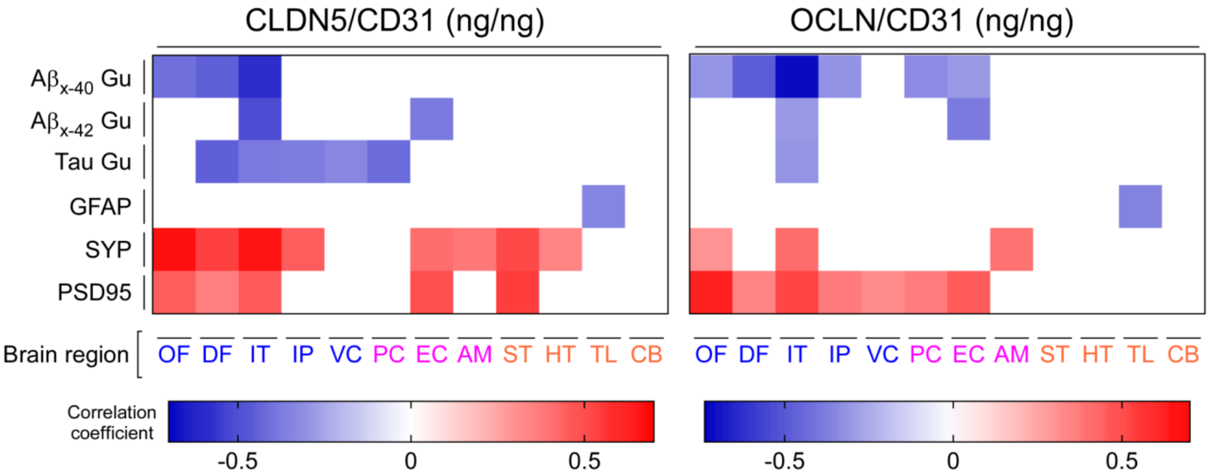
Yamazaki et al., submitted

Brain region-dependent correlations between Braak stage and TJ proteins



Yamazaki et al., submitted

Brain region-dependent correlations between TJ proteins and AD-related molecules



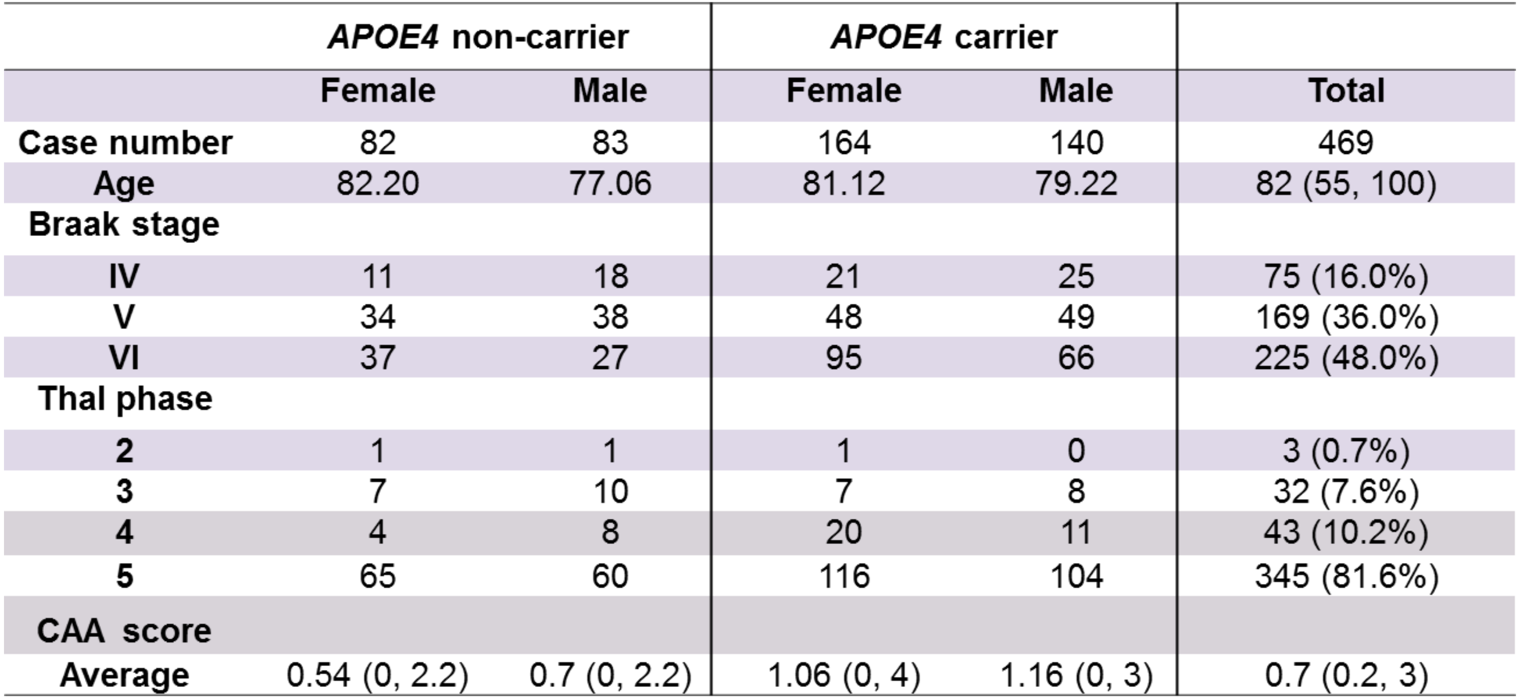
	CLDN5/CD31		OCLN/CD31	
	Synaptophysin	PSD95	Synaptophysin	PSD95
A$\beta_{x\text{-}40}$	-0.71 (0.4798)	-0.72 (0.4752)	-0.89 (0.3779)	-0.93 (0.359)
A$\beta_{x\text{-}42}$	-1.14 (0.2605)	-0.92 (0.3633)	-1.26 (0.2160)	-1.09 (0.2836)
Tau	-0.11 (0.9954)	0.30 (0.7650)	0.12 (0.9035)	0.43 (0.6667)
TJs/CD31	1.12 (0.2695)	**2.43 (0.0201)**	0.67 (0.5089)	**2.04 (0.0491)**

Yamazaki et al., submitted

Human postmortem brain cohort 2: effects of *APOE4* and sex

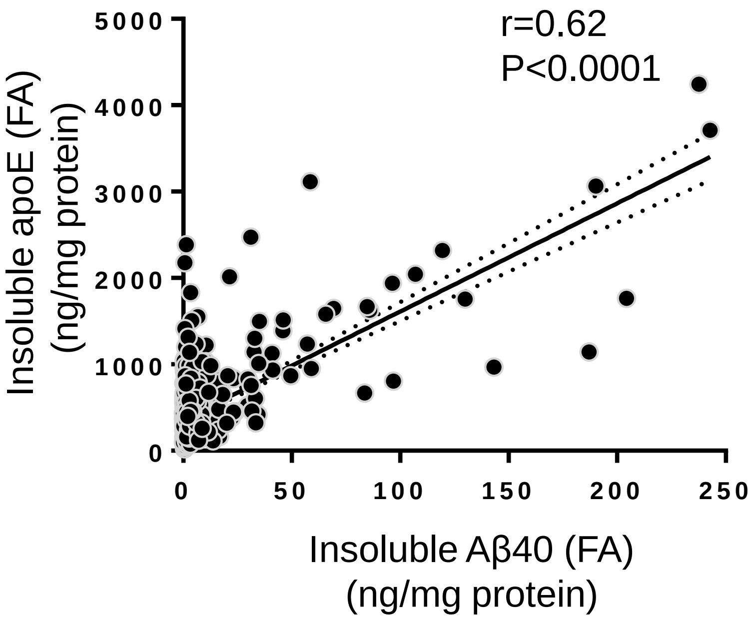
(Adjacent to brain sections for genomics and transcriptomics by Ertekin-Taner Lab)

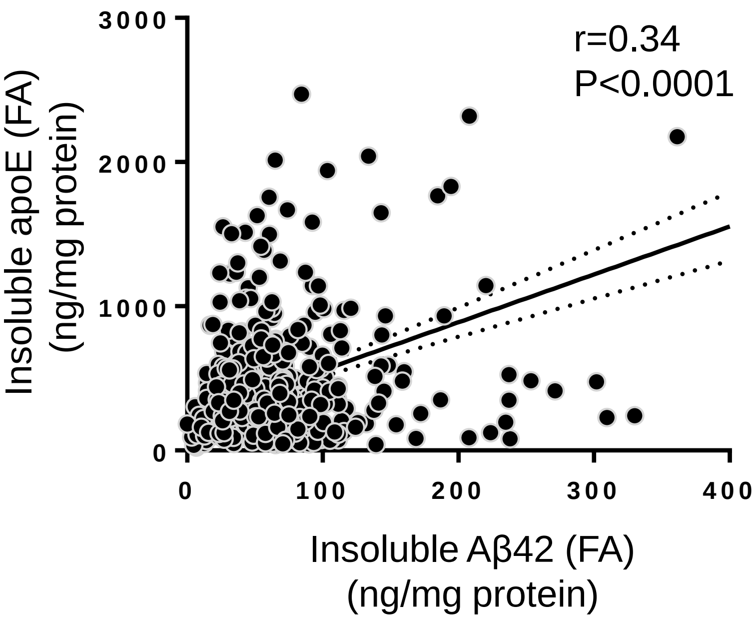
Subject characteristics of AD brain samples



	APOE4 non-carrier		*APOE4* carrier		
	Female	**Male**	**Female**	**Male**	**Total**
Case number	82	83	164	140	469
Age	82.20	77.06	81.12	79.22	82 (55, 100)
Braak stage					
IV	11	18	21	25	75 (16.0%)
V	34	38	48	49	169 (36.0%)
VI	37	27	95	66	225 (48.0%)
Thal phase					
2	1	1	1	0	3 (0.7%)
3	7	10	7	8	32 (7.6%)
4	4	8	20	11	43 (10.2%)
5	65	60	116	104	345 (81.6%)
CAA score					
Average	0.54 (0, 2.2)	0.7 (0, 2.2)	1.06 (0, 4)	1.16 (0, 3)	0.7 (0.2, 3)

Insoluble apoE is positively correlated with insoluble Aβ





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Tight junction molecule CLDN5 is negatively associated with apoE and Aβ42

	***APOE4* non-carrier**		***APOE4* carrier**	
	CLDN5/CD31		CLDN5/CD31	
	r	p value	*r*	p value
ApoE TBS	-0.279	0.0003	-0.258	<.0001
ApoE TX	-0.199	0.0108	-0.155	0.0072
ApoE FA	-0.219	0.0049	-0.087	0.1324
Aβ40 TBS	-0.094	0.2294	-0.029	0.6193
Aβ40 TX	-0.079	0.3145	-0.006	0.9242
Aβ40 FA	-0.077	0.3271	-0.017	0.7675
Aβ42 TBS	-0.175	0.0254	-0.091	0.116
Aβ42 TX	0.135	0.0841	-0.101	0.0821
Aβ42 FA	-0.054	0.4921	0.021	0.7213

	Female		**Male**	
	CLDN5/CD31		CLDN5/CD31	
	r	p value	*r*	p value
ApoE TBS	-0.264	<.0001	-0.309	<.0001
ApoE TX	-0.198	0.0018	-0.132	0.0508
ApoE FA	-0.151	0.0177	-0.146	0.0289
Aβ40 TBS	-0.052	0.4145	-0.064	0.3416
Aβ40 TX	-0.031	0.6236	-0.071	0.2879
Aβ40 FA	-0.067	0.2931	-0.034	0.6085
Aβ42 TBS	-0.137	0.0313	-0.131	0.0505
Aβ42 TX	-0.063	0.3242	-0.044	0.5103
Aβ42 FA	-0.065	0.307	0.012	0.8586

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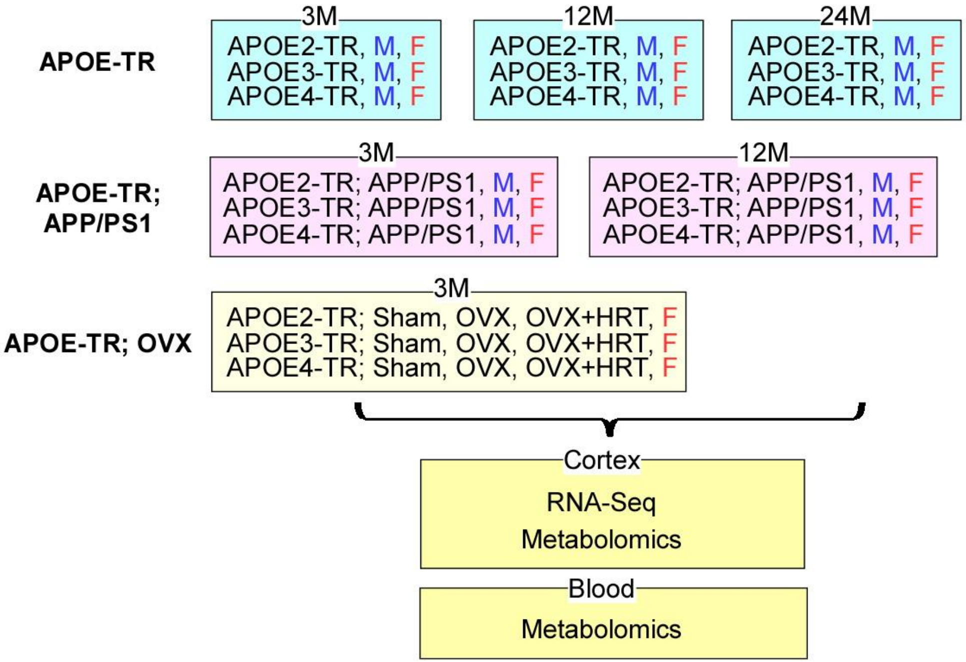
Identification of transcriptome/metabolome signatures associated with *APOE*, sex and aging using systems-based, non-targeted approaches



APOE-TR

3M
APOE2-TR, M, F
APOE3-TR, M, F
APOE4-TR, M, F

12M
APOE2-TR, M, F
APOE3-TR, M, F
APOE4-TR, M, F

24M
APOE2-TR, M, F
APOE3-TR, M, F
APOE4-TR, M, F

APOE-TR; APP/PS1

3M
APOE2-TR; APP/PS1, M, F
APOE3-TR; APP/PS1, M, F
APOE4-TR; APP/PS1, M, F

12M
APOE2-TR; APP/PS1, M, F
APOE3-TR; APP/PS1, M, F
APOE4-TR; APP/PS1, M, F

APOE-TR; OVX

3M
APOE2-TR; Sham, OVX, OVX+HRT, F
APOE3-TR; Sham, OVX, OVX+HRT, F
APOE4-TR; Sham, OVX, OVX+HRT, F

Cortex
RNA-Seq
Metabolomics

Blood
Metabolomics

- ○ **8 brain and serum samples/group/sex**

- ○ **RNA-Seq**
- • Mayo Clinic Medical Genome Facility

- ○ **Metabolomics**
- • Duke University Medical Center-Dr. Rima Kaddurah-Daouk

- ○ **Network analysis**
- • WGCNA, multi-omics data integration and others (Mayo and Mount Sinai Bioinformatics Teams)

Human APOE-targeted replacement (TR) mice by Patrick Sullivan and colleagues at Duke

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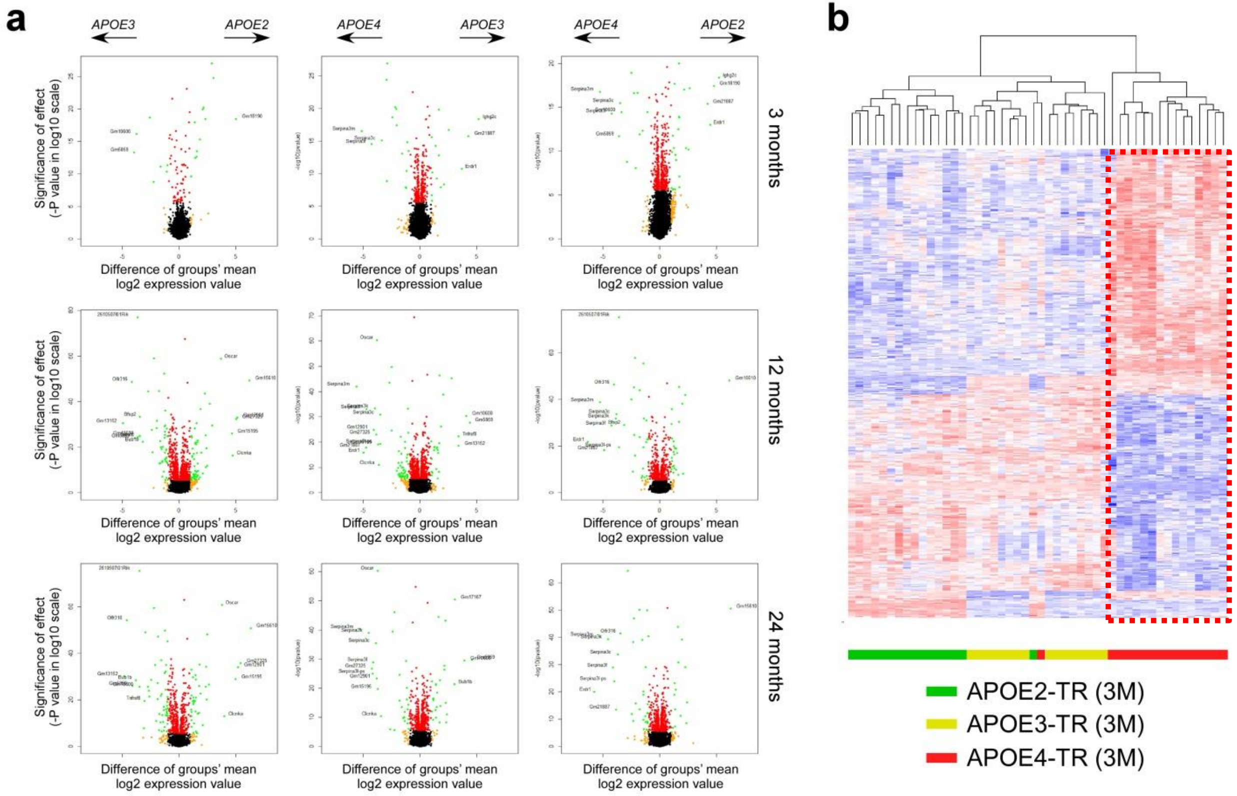
Volcano plots and hierarchical clustering of DEGs showing distinct transcriptomic signatures in *APOE4*-TR mice



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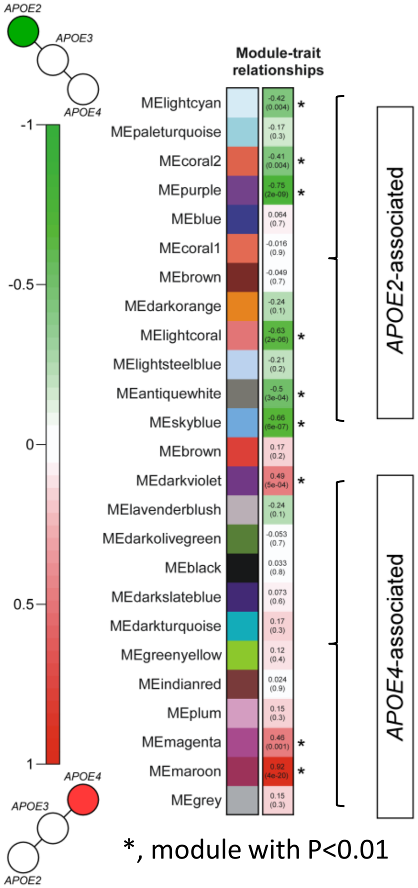
(n = 16/genotype/age, M/F ratio = 1)

Bioinformatics identification of AD-relevant *APOE* genotype effectors



Module-trait relationships

MElightcyan	-0.42 (0.004)	*
MEpaleturquoise	-0.17 (0.3)	
MEcoral2	-0.41 (0.004)	*
MEpurple	-0.75 (2e-09)	*
MEblue	0.064 (0.7)	
MEcoral1	-0.016 (0.9)	
MEbrown	-0.049 (0.7)	
MEdarkorange	-0.24 (0.1)	
MElightcoral	-0.63 (2e-06)	*
MElightsteelblue	-0.21 (0.2)	
MEantiquewhite	-0.5 (3e-04)	*
MEskyblue	-0.66 (6e-07)	*
MEbrown	0.17 (0.2)	
MEdarkviolet	0.49 (5e-04)	*
MElavenderblush	-0.24 (0.1)	
MEdarkolivegreen	-0.053 (0.7)	
MEblack	0.033 (0.8)	
MEdarkslateblue	0.073 (0.6)	
MEdarkturquoise	0.17 (0.3)	
MEgreenyellow	0.12 (0.4)	
MEindianred	0.024 (0.9)	
MEplum	0.15 (0.3)	
MEmagenta	0.46 (0.001)	*
MEmaroon	0.92 (4e-20)	*
MEgrey	0.15 (0.3)	

*, module with P<0.01

Synaptic transmission, neurogenesis and neuronal projection

GO terms enriched in module (logP)

ATP binding (-4)
adenyl ribonucleotide binding (-3)
adenyl nucleotide binding (-3)
phospholipid biosynthetic process (-3)

neurogenesis (-6)
generation of neurons (-6)
nervous system development (-5)
cell periphery (-5)
neuron differentiation (-5)
axon development (-5)
receptor complex (-5)
signaling (-5)
cell development (-5)
plasma membrane (-5)

N/A

histone modification (-7)
microtubule cytoskeleton (-7)
microtubule organizing center (-6)
nucleoplasm (-6)
chromatin modification (-6)
mRNA processing (-6)
chromatin organization (-6)
centrosome (-6)
nuclear lumen (-5)
cilium organization (-5)

neuron projection (-3)

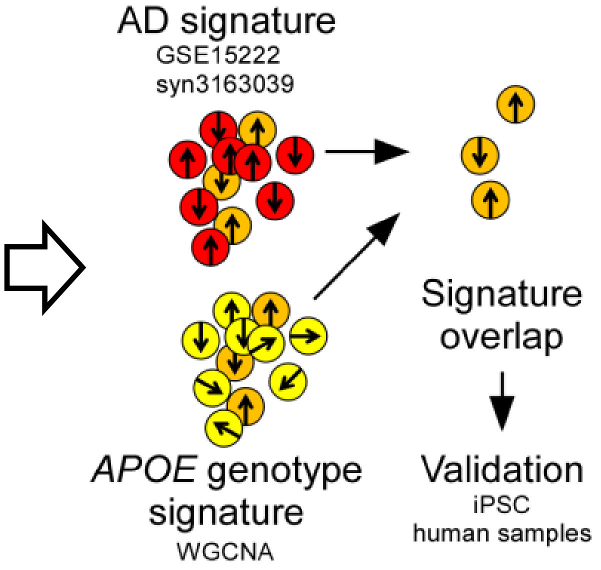
N/A

RNA binding (-18)
poly(A) RNA binding (-18)
intracellular ribonucleoprotein complex (-17)
ribonucleoprotein complex (-17)
ribosome (-14)
nucleic acid binding (-13)
ribosomal subunit (-12)
RNA processing (-11)
intracellular membrane-bounded organelle (-11)
RNA splicing (-10)

integral component of membrane (-10)
intrinsic component of membrane (-10)
extracellular region (-8)
vesicle (-8)
fatty acid beta-oxidation (-7)
lipid oxidation (-7)
membrane-bounded vesicle (-7)
oxidation-reduction process (-7)
cell surface (-7)

response to interferon-beta (-9)
RNA binding (-8)
cellular response to interferon-beta (-8)
membrane-bounded organelle (-7)
nucleus (-7)
nuclear part (-6)
intracellular membrane-bounded organelle (-6)
poly(A) RNA binding (-6)
ligase activity, forming aminoacyl-tRNA and related compounds (-6)
tRNA aminoacylation for protein translation (-6)

RNA processing, metabolism, lipid oxidation and inflammation



AD signature
GSE15222
syn3163039

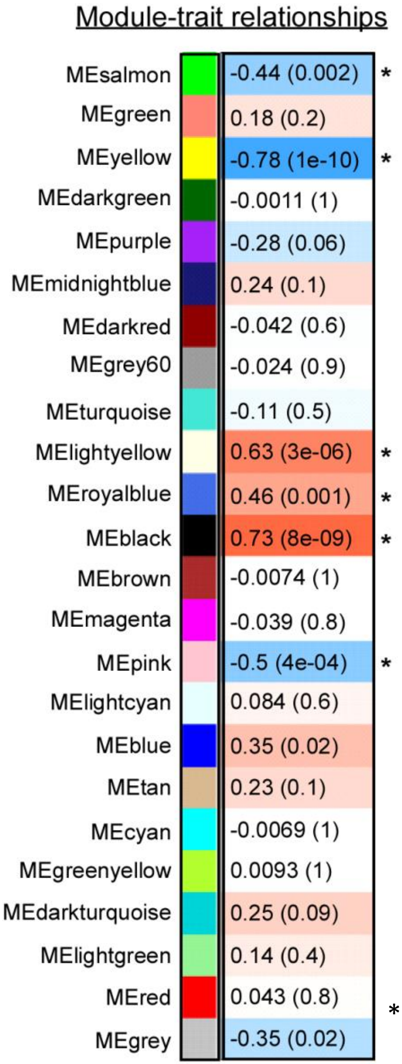
APOE genotype signature
WGCNA

Signature overlap

Validation
iPSC
human samples

(**3 months**, n = 16/genotype/age, M/F ratio = 1)

Immune response pathways are upregulated in APOE4-TR mice at 12 months of age



Module-trait relationships

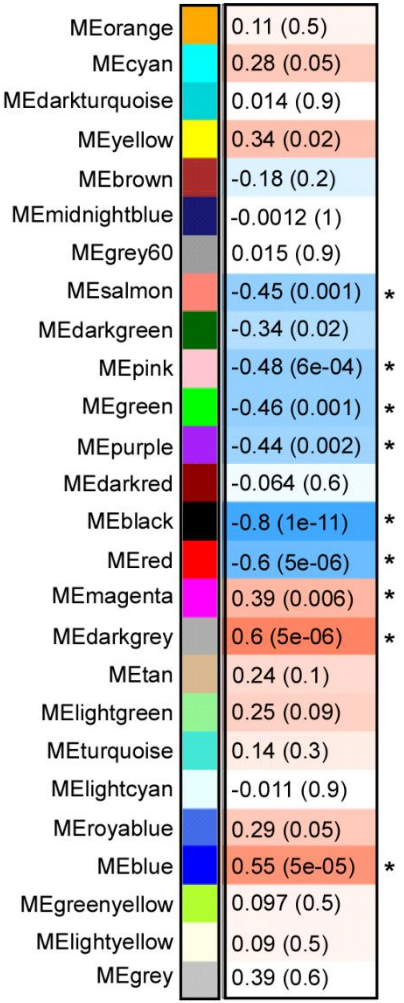
Module	Correlation (P)	
MEsalmon	-0.44 (0.002)	*
MEgreen	0.18 (0.2)	
MEyellow	-0.78 (1e-10)	*
MEdarkgreen	-0.0011 (1)	
MEpurple	-0.28 (0.06)	
MEmidnightblue	0.24 (0.1)	
MEdarkred	-0.042 (0.6)	
MEgrey60	-0.024 (0.9)	
MEturquoise	-0.11 (0.5)	
MElightyellow	0.63 (3e-06)	*
MEroyalblue	0.46 (0.001)	*
MEblack	0.73 (8e-09)	*
MEbrown	-0.0074 (1)	
MEmagenta	-0.039 (0.8)	
MEpink	-0.5 (4e-04)	*
MElightcyan	0.084 (0.6)	
MEblue	0.35 (0.02)	
MEtan	0.23 (0.1)	
MEcyan	-0.0069 (1)	
MEgreenyellow	0.0093 (1)	
MEdarkturquoise	0.25 (0.09)	
MElightgreen	0.14 (0.4)	
MEred	0.043 (0.8)	
MEgrey	-0.35 (0.02)	

*, module with P<0.01

Rank	GO term ID	Term name
1	GO:0002376	immune system process
2	GO:0002682	regulation of immune system process
3	GO:0006955	immune response
4	GO:0002443	leukocyte mediated immunity
5	GO:0002699	positive regulation of immune effector process
6	GO:0050900	leukocyte migration
7	GO:0050776	regulation of immune response
8	GO:0002685	regulation of leukocyte migration
9	GO:0030595	leukocyte chemotaxis
10	GO:0002684	positive regulation of immune system process

(12 months, n = 16/genotype, M/F ratio = 1)

Immune response pathways are upregulated in APOE4-TR mice at <u>24 months</u> of age



Module-trait relationships

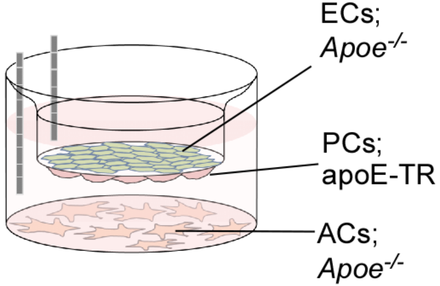
Module	Correlation (P)	
MEorange	0.11 (0.5)	
MEcyan	0.28 (0.05)	
MEdarkturquoise	0.014 (0.9)	
MEyellow	0.34 (0.02)	
MEbrown	-0.18 (0.2)	
MEmidnightblue	-0.0012 (1)	
MEgrey60	0.015 (0.9)	
MEsalmon	-0.45 (0.001)	*
MEdarkgreen	-0.34 (0.02)	
MEpink	-0.48 (6e-04)	*
MEgreen	-0.46 (0.001)	*
MEpurple	-0.44 (0.002)	*
MEdarkred	-0.064 (0.6)	
MEblack	-0.8 (1e-11)	*
MEred	-0.6 (5e-06)	*
MEmagenta	0.39 (0.006)	*
MEdarkgrey	0.6 (5e-06)	*
MEtan	0.24 (0.1)	
MElightgreen	0.25 (0.09)	
MEturquoise	0.14 (0.3)	
MElightcyan	-0.011 (0.9)	
MEroyablue	0.29 (0.05)	
MEblue	0.55 (5e-05)	*
MEgreenyellow	0.097 (0.5)	
MElightyellow	0.09 (0.5)	
MEgrey	0.39 (0.6)	

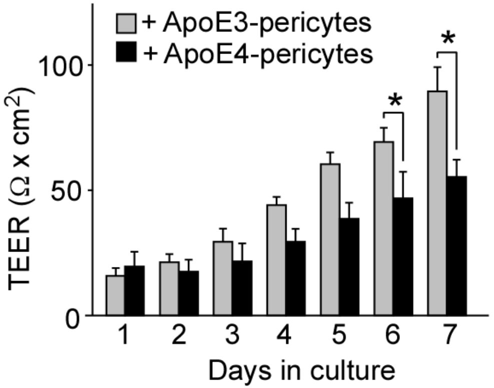
*, module with P<0.01

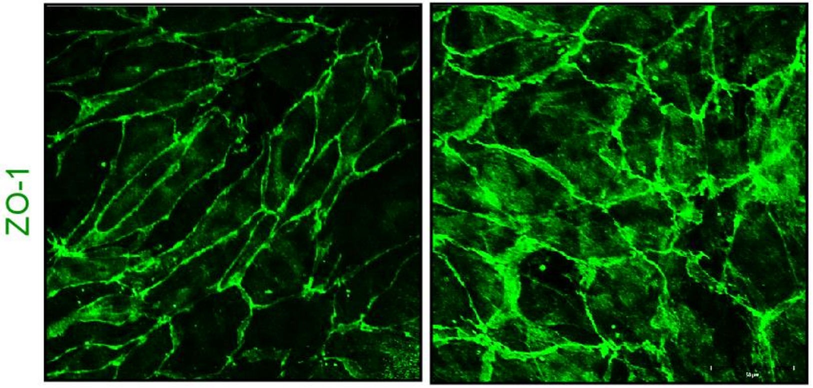
Rank	GO term ID	Term name
1	GO:0045087	innate immune response
2	GO:0006955	immune response
3	GO:0006952	defense response
4	GO:0098542	defense response to other organism
5	GO:0051707	response to other organism
6	GO:0009607	response to biotic stimulus
7	GO:0002376	immune system process
8	GO:0009615	response to virus
9	GO:0051607	defense response to virus
10	GO:0035456	response to interferon-beta

(**24 months**, n = 16/genotype, M/F ratio = 1)

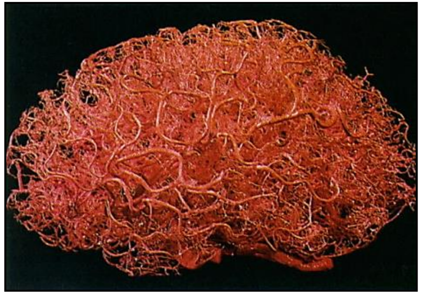
ApoE in cerebrovasculature: ApoE4-pericytes are less efficient in supporting endothelial barrier formation in an *in vitro* BBB model







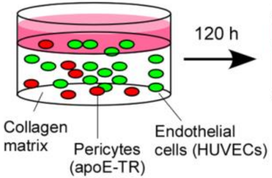
+ ApoE3-pericytes   + ApoE4-pericytes

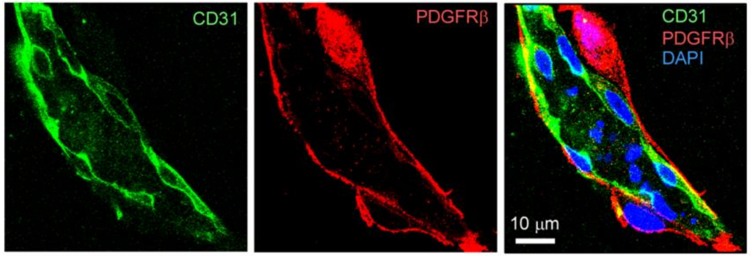


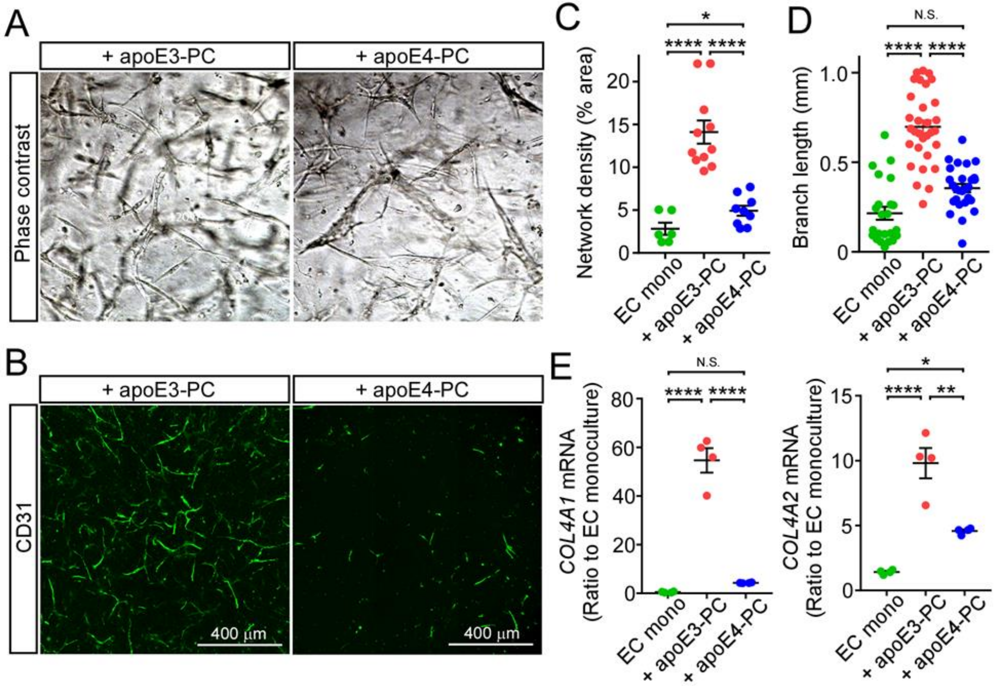
ApoE is abundantly expressed in vascular mural cells: SMC and pericytes

Yamazaki et al., In revision

ApoE4-pericytes are less efficient in stimulating endothelial lumenogenesis in a 3-D co-culture system

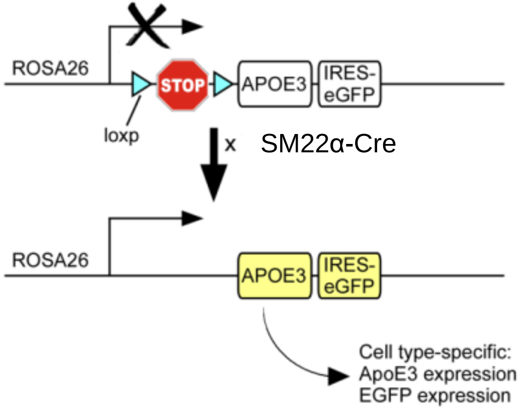


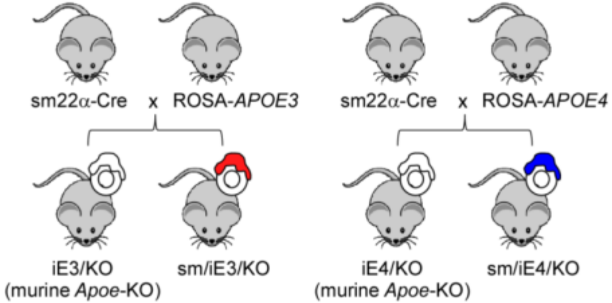


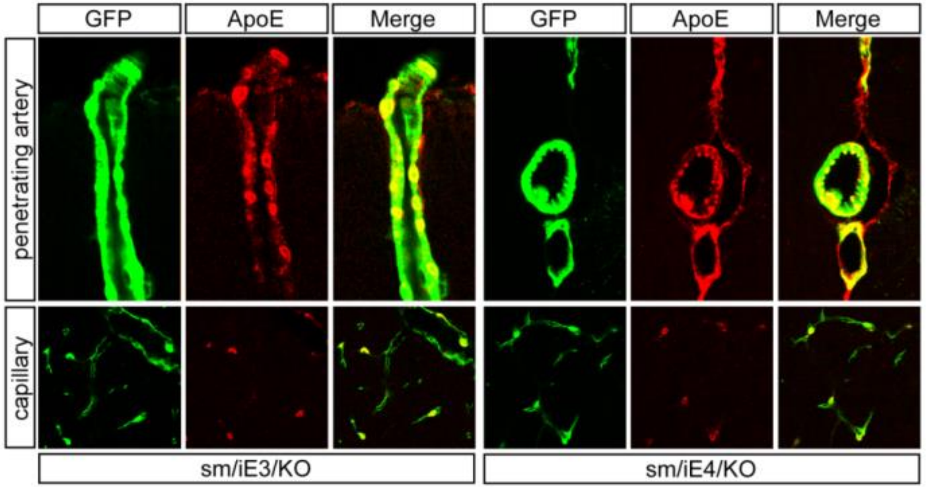


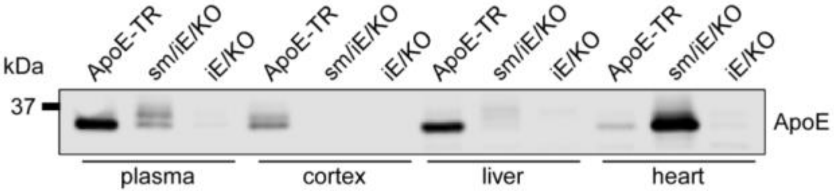
Yamazaki et al., In revision

Conditional expression of human apoE3 or apoE4 in VMCs using SM22α-Cre in *Apoe*-KO background


ROSA26
STOP
APOE3
IRES-eGFP
loxp
x SM22α-Cre
ROSA26
APOE3
IRES-eGFP
Cell type-specific:
ApoE3 expression
EGFP expression

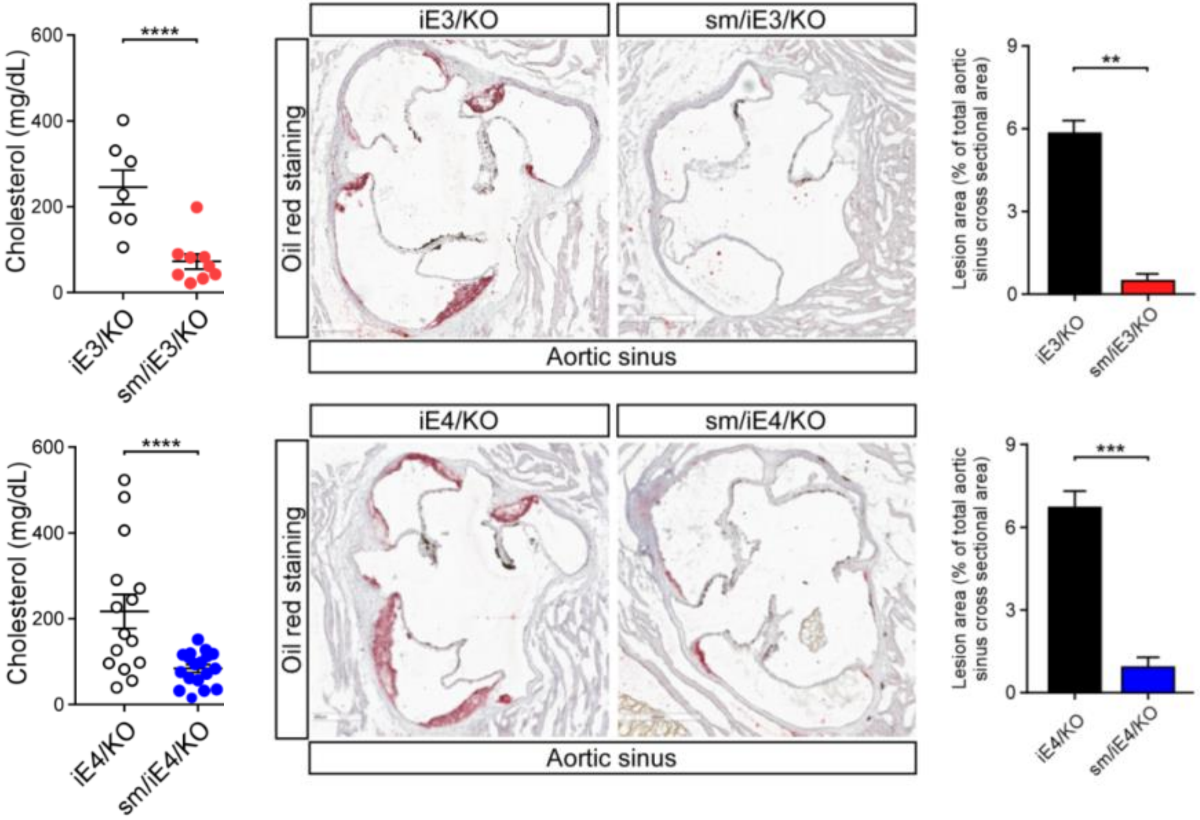

sm22α-Cre x ROSA-*APOE3*
sm22α-Cre x ROSA-*APOE4*
iE3/KO (murine *Apoe*-KO)
sm/iE3/KO
iE4/KO (murine *Apoe*-KO)
sm/iE4/KO





Yamazaki et al., unpublished

Conditional expression of human apoE3 or E4 in VMCs rescues lipid and atherosclerotic phenotypes resulted from murine *Apoe* deficiency

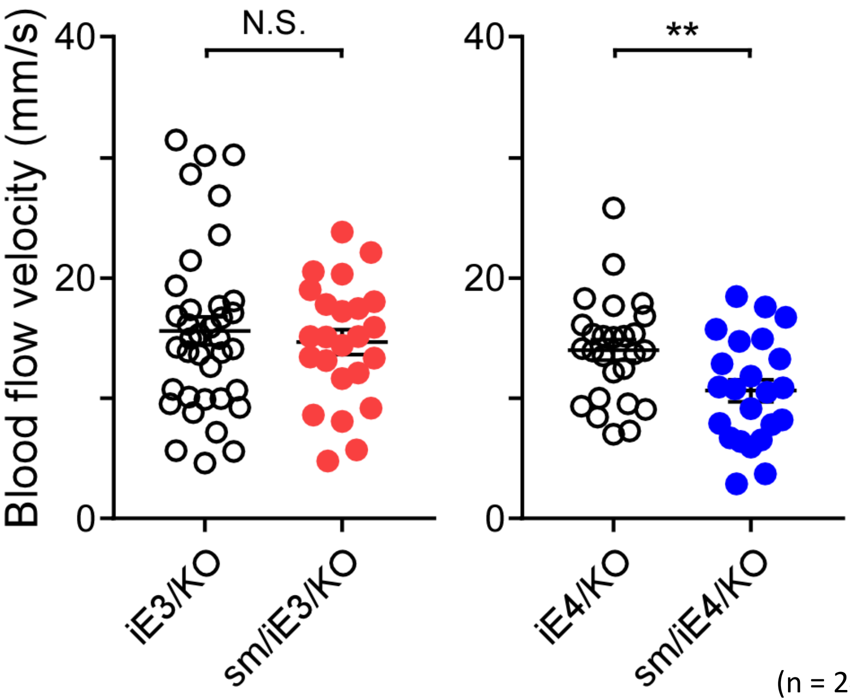


Data are presented as mean ± s.e.m. ****$P < 0.0001$, ***$P < 0.001$ and **$P < 0.01$, t-test

Yamazaki et al., unpublished

MAYO
CLINIC

Arteriole blood flow measured by *in vivo* 2-photon imaging is decreased in sm/iE4/KO mice

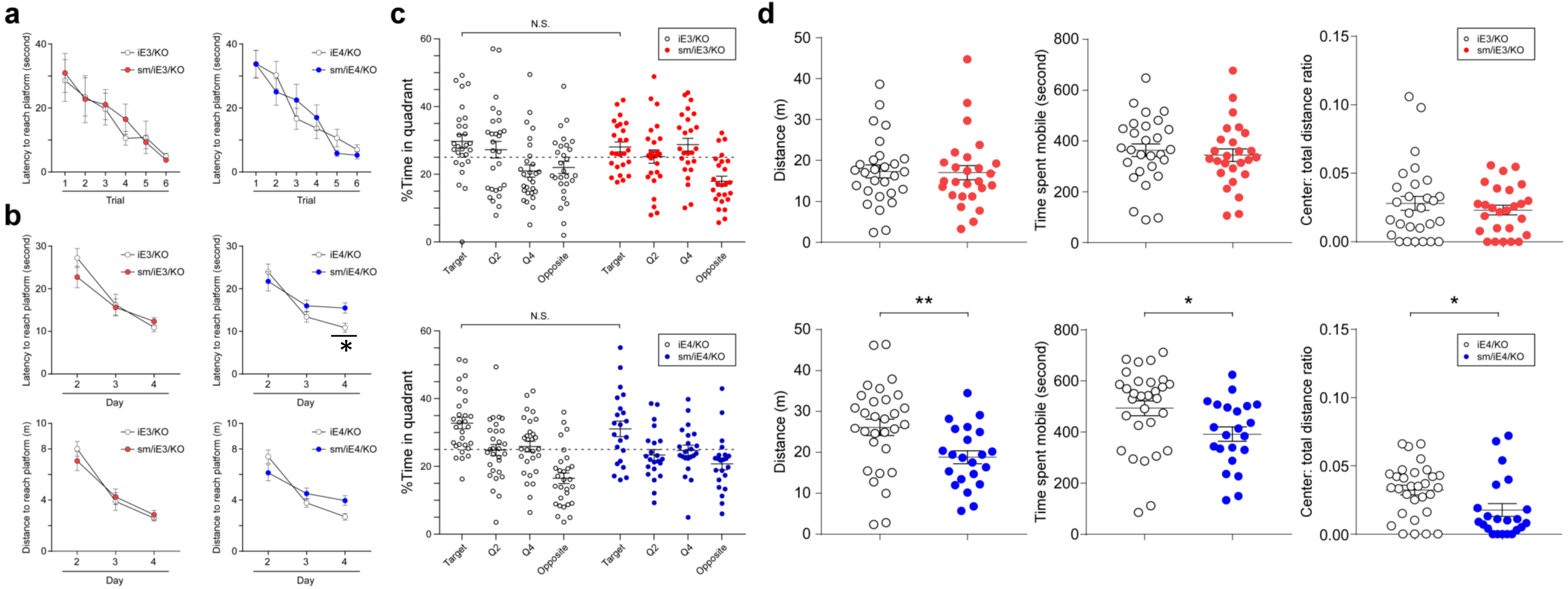


(n = 24 -48 arterioles from 3 mice/group)

Data are presented as mean ± s.e.m. $^{**}P < 0.01$, t-test. N.S., not significant.

Yamazaki et al., unpublished

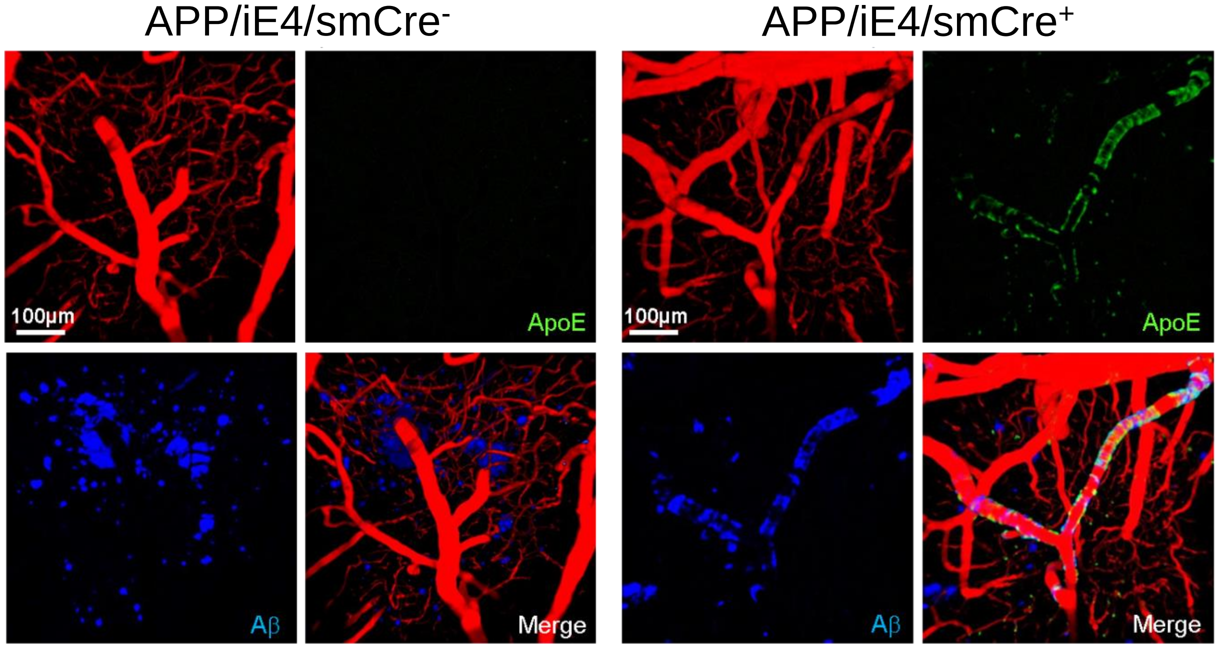
Conditional expression of apoE4 in VMCs leads to increased anxiety-like behavior and impaired spatial learning



Data are presented as mean ± s.e.m. **$P < 0.01$ and *$P < 0.05$, t-test. N.S., not significant.

Yamazaki et al., unpublished

Expression of apoE4 in vascular mural cells redistributed Aβ into the vasculature as CAA



- SM22α-Cre, vascular-specific in *Apoe*-KO background
- Bred to APP/PS1 amyloid model mice
- Two-photon images (collaboration with Betty Kim lab)
- Vascular apoE seeds CAA

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Liu et al., unpublished

Summary

Postmortem brain studies

- *APOE4* and male sex are correlated with increased severity of CAA in AD.
- Aβ40 and Aβ40/Aβ42 are selectively increased in AD brains with CAA in a sex- and *APOE4*-dependent manner
- Endothelial tight junction markers are progressively decreased in AD.

Animal model omics studies

- Transcriptomic signature in *APOE4*-TR mice is distinct from that of *APOE2*-TR or *APOE3*-TR mice at different ages.
- AD relevance of *APOE* genotype-related signatures will be determined through a comparison with human transcriptomic datasets available from the AMP-AD Knowledge Portals.
- Identified AD-relevant "APOE effectors" will further be validated using iPSC and human samples (i.e., CSF, postmortem brains).

In vitro and *in vivo* functional studies

- ApoE in pericytes regulates endothelial expression of ECM in an isoform-dependent manner, which influences vasculogenesis in a 3-D culture system.
- Conditional expression of apoE4 in VMCs leads to an increased anxiety-like phenotype, impaired learning, and reduced arteriolar blood flow.
- Expression of apoE4 in vascular mural cells redistributed Aβ into the vasculature as CAA.

MAYO CLINIC

Data either are deposited or will be deposited shortly at https://www.synapse.org

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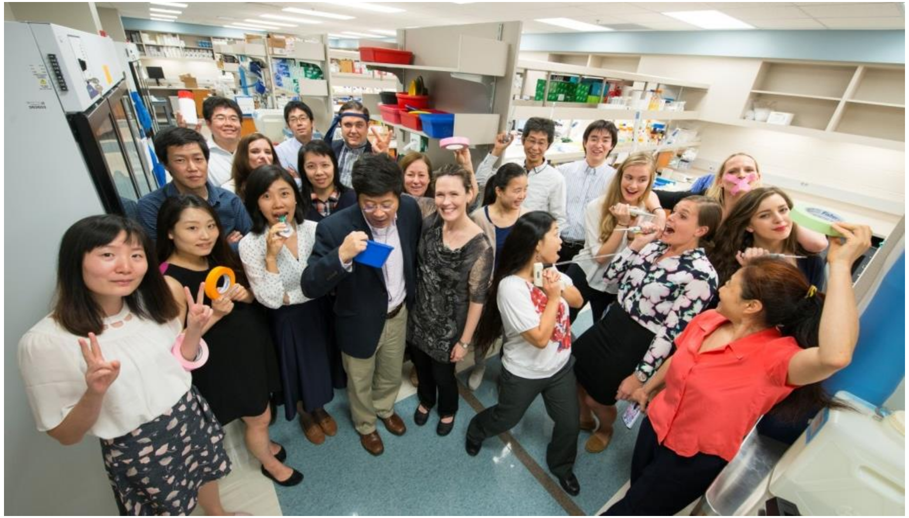

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