21st US-Japan Cellular and Gene Therapy Conference
Neurodegenerative Diseases: Biology, Cellular and Gene Therapy

FDA White Oak Campus, Building 31, Great Room, 1503 A
Silver Spring, Maryland 20993

Thursday, March 1, 2018
9:00 am – 5:00 pm

Tatsuhiro Hisatsune
Graduate School of Frontier Sciences
The University of Tokyo, Japan

Rise in the Number of Dementia Patients

Japan

2014 **4.6 million**

Seven Years

2025 **7.0 million**

(Estimation)

https://www.asahi.com/articles/ASK4R7J67K4RUBQU00S.html

World

2015 **46 million**

Thirty Years **Three Times**

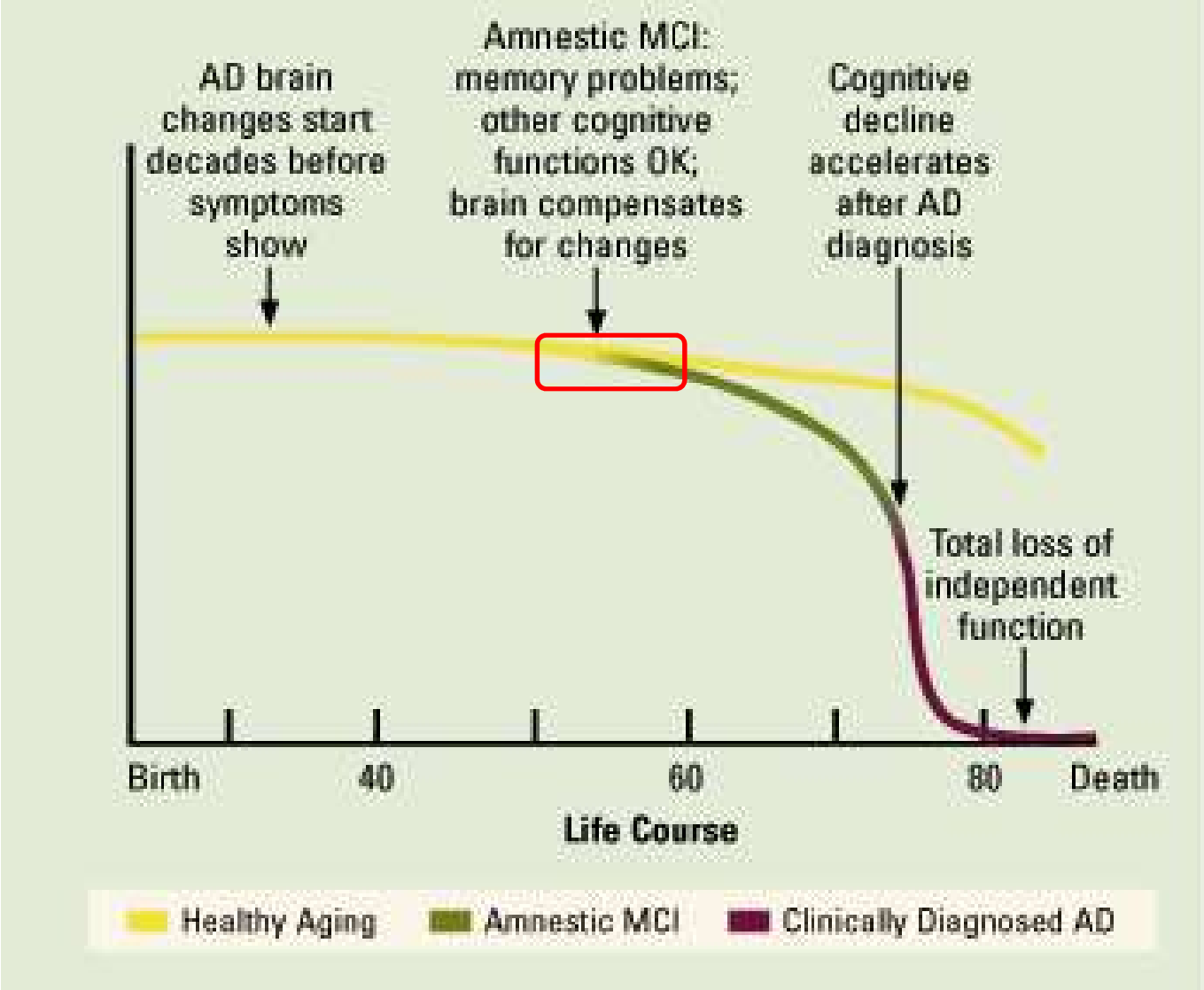
2050 **132 million**

(Estimation)

http://www.worldalzreport2015.org/

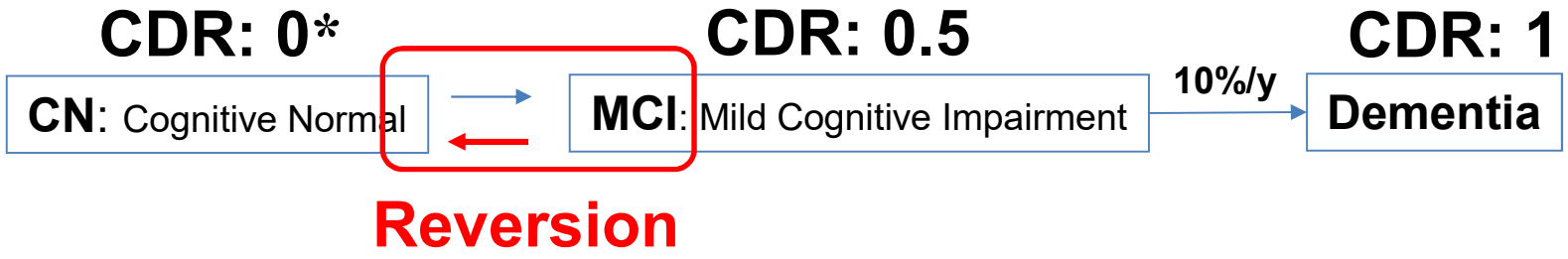
http://www.who.int/mental_health/neurology/dementia/dementia_thematicbrief_epidemiology.pdf

The Course of Healthy Aging, Mild Cognitive Impairment (MCI), and Alzheimer's Disease (AD)



http://www.nia.nih.gov/Alzheimers/Publications/ADProgress2004_2005/

Cognitive function of elderly people (≥ 65 years-old)



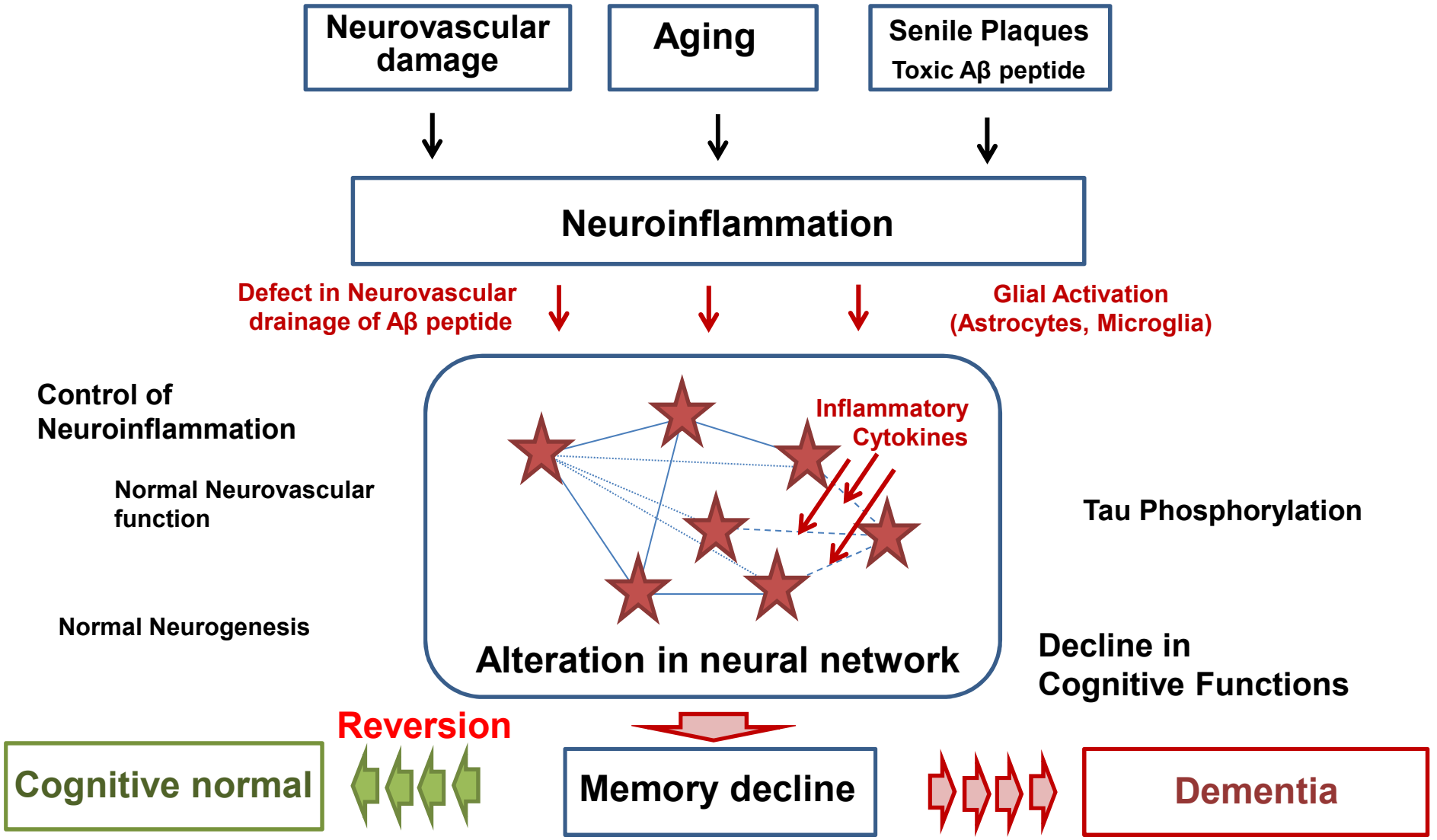
Cognitive function (DSM-5)*

	MCI due to AD	AD
Learning & memory	Slight Decline	
Language	Normal	
Executive function	Normal	Definite Decline
Social cognition	Normal	
Complex attention	Normal	
Perceptual-motor	Normal	

http://www.alz.org/jp

- CDR: Clinical Dementia Rating
- DSM-5: Diagnostic and Statistical Manual of Mental Disorders-5th version

Neuroinflammation: as a target of regenerative medication for MCI



Regenerative medication for the prevention of the onset of Dementia

Strategies to control neuroinflammation

Many trials with:

- **Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)**

  NSAIDs, such as ibuprofen and indomethacin

  COX-2 inhibitors, such as rofecoxib and celecoxib

- **To control inflammatory cytokines**

  Antagonists, Specific blockers

  Antibodies toward inflammatory cytokines

- **Anti-inflammatory small molecules**

  → Anti-inflammatory drugs, such as ChE inhibitors

  ➡ Anti-inflammatory natural products

Ref “Can Alzheimer’s Disease Be Prevented?” NIH Publication No. 09-5503, April 2009

Age-related declines in Cognitive Functions in Animal and Human

1995 2000 2005 2010 2015 2020

Mouse

Adult Hippocampal Neurogenesis

NIH
NINDS

Stroke model

Aging study

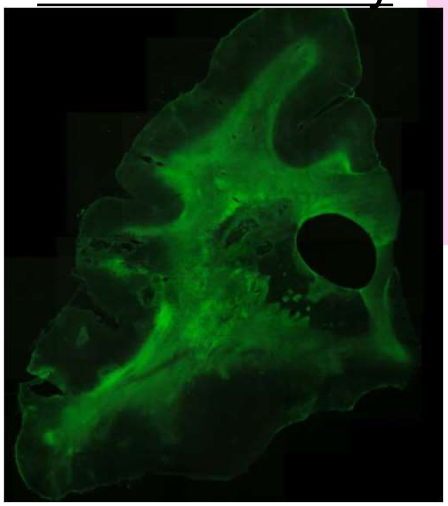
Alzheimer's Disease Model Mouse

Monkey

Adult Neurogenesis

Stroke

Stroke monkey



Sato et al. (2009)

Aging study

Mild Cognitive Impairment

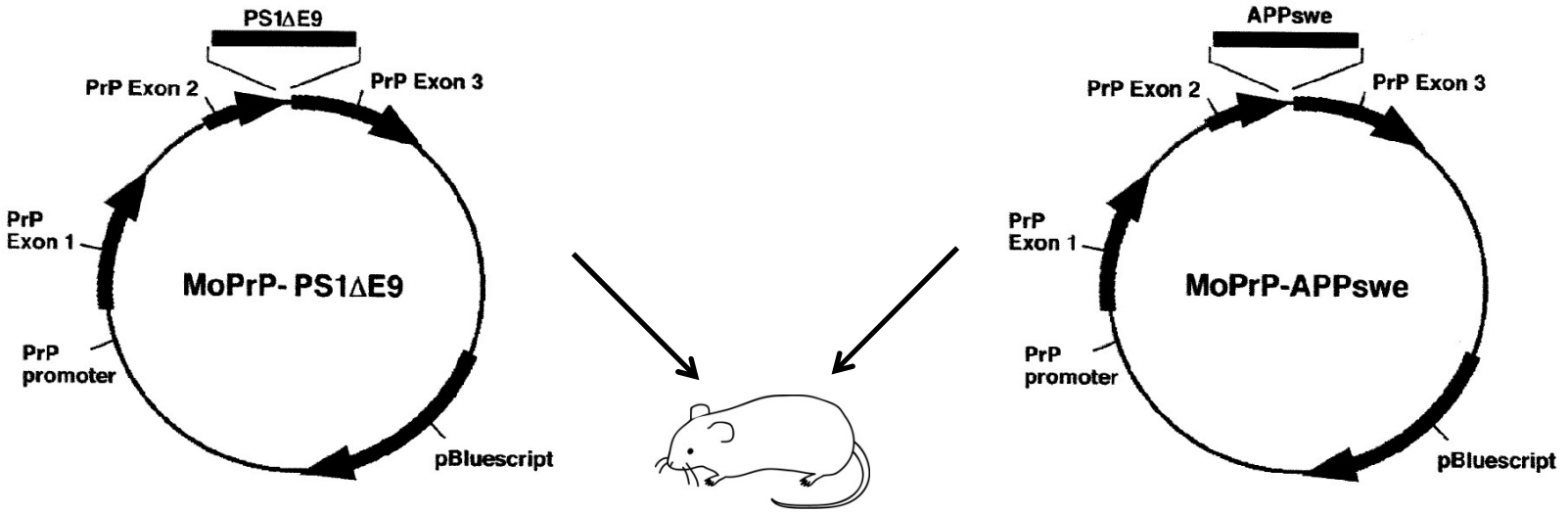
Alzheimer's Disease

Human

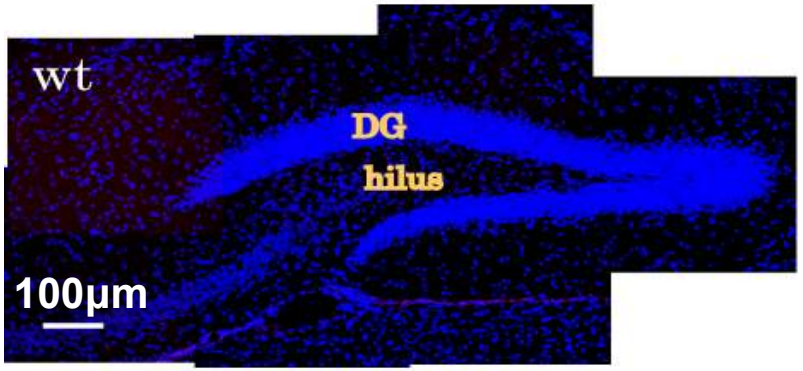
Neuroinflammation <Glial Activation>

Alzheimer's Disease (AD) Model Mouse

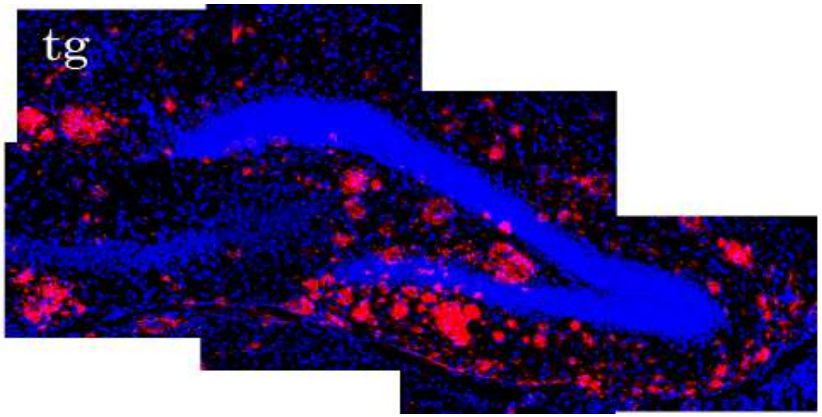
【B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J】



Wild Type



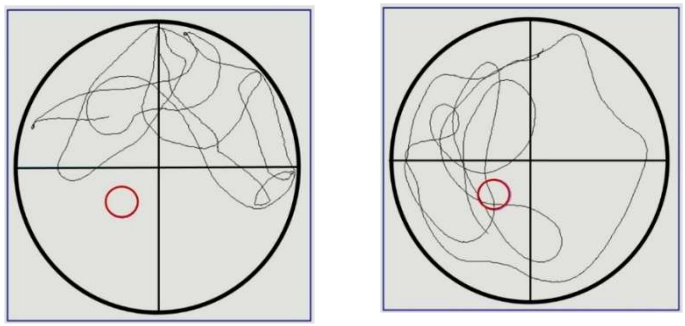
AD Transgenic

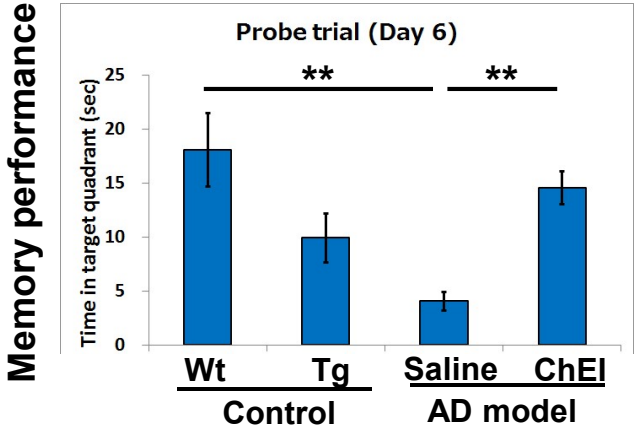


Senile Plaques (18 month olds: Red, Anti-Aβ staining)

Control of memory decline and neuroinflammation by Choline-esterase inhibitor (ChEI)

Water Maze (Probe test)

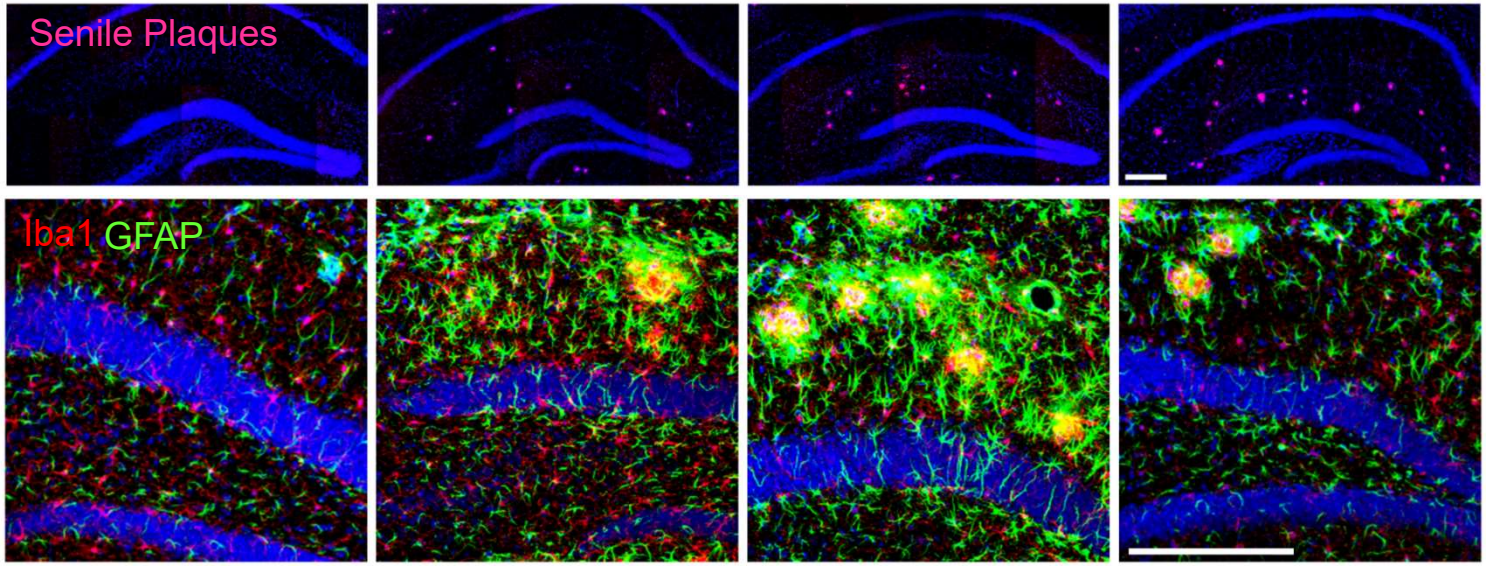




ChEI has:

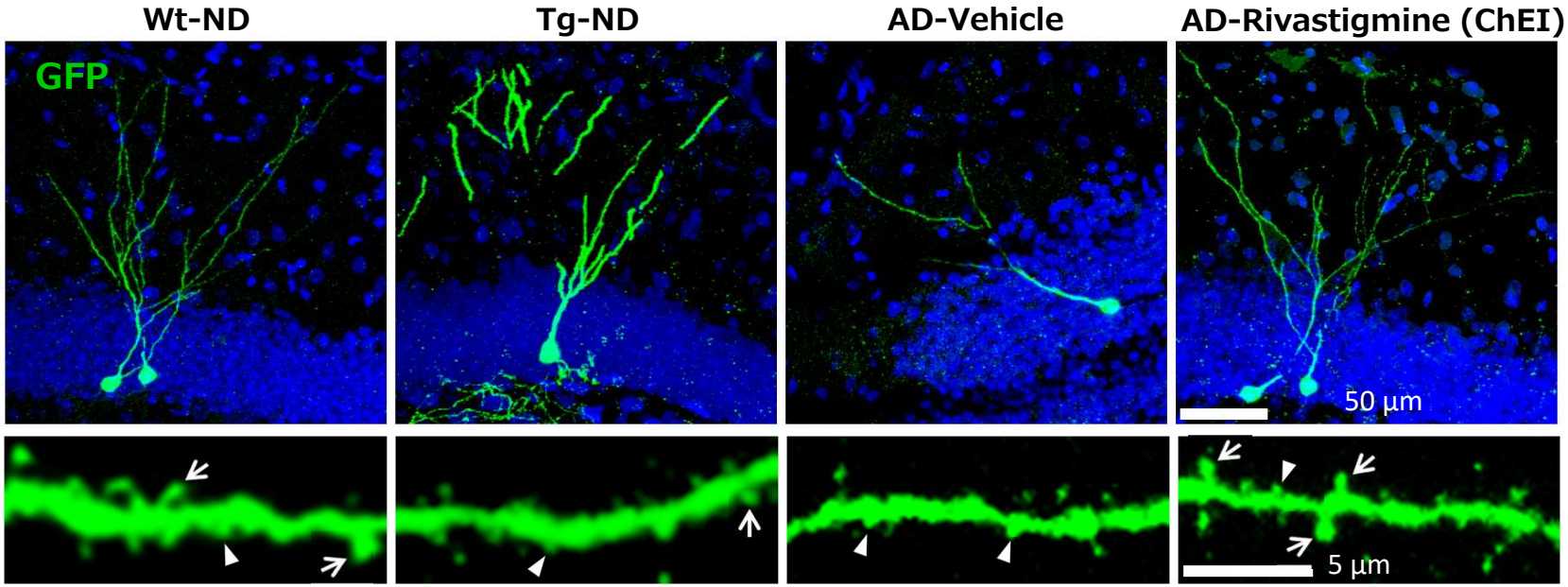
no effect on plaque formation

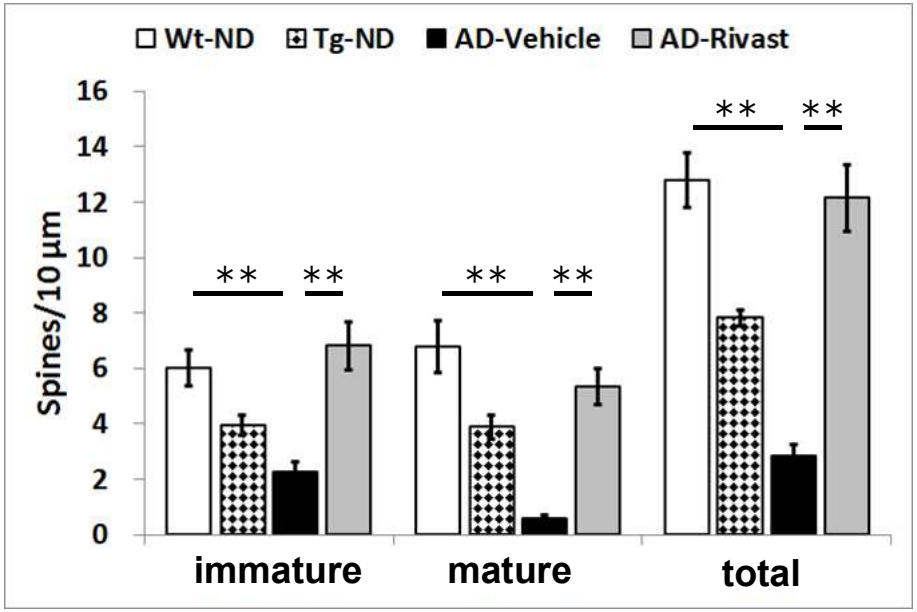
suppressive effect on neuroinflammation

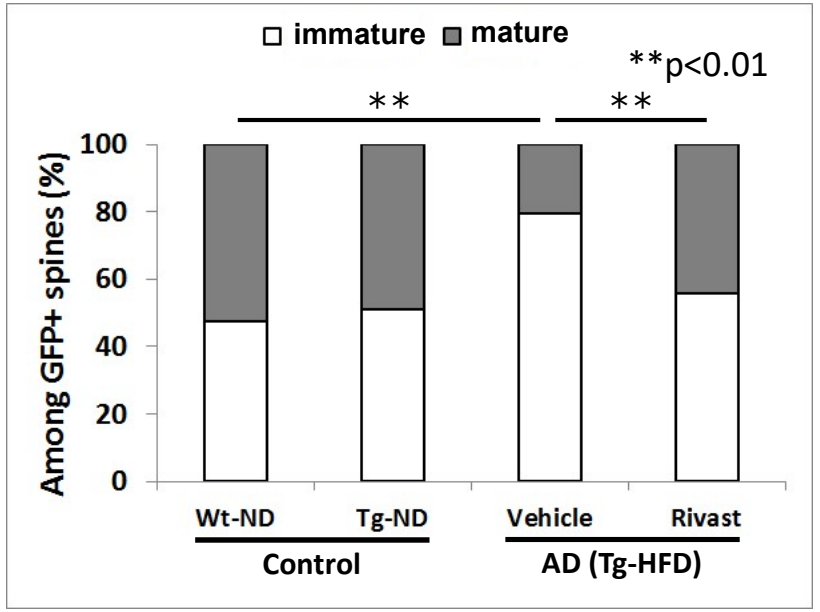


Matsuda et al. J Alzheimer`s Disease (2017)

ChEI Recovers Adult Neurogenesis in AD model mouse



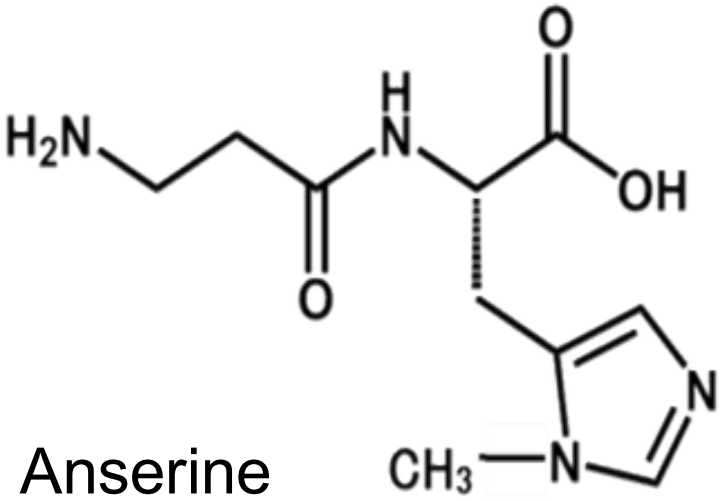




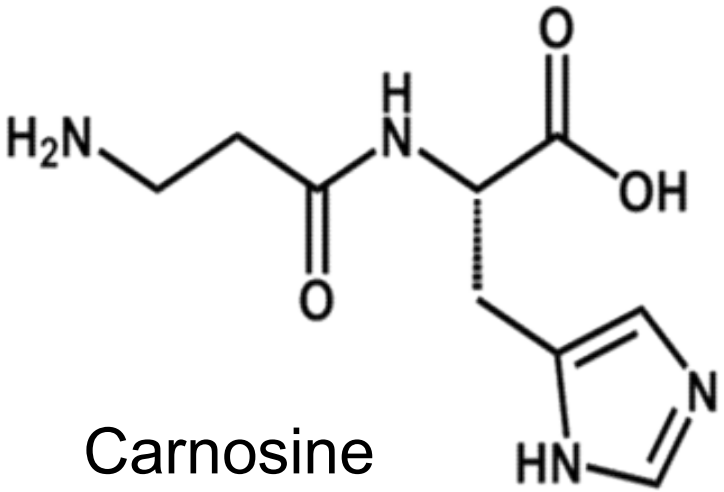
Matsuda et al. J Alzheimer`s Disease (2017)

Imidazole-di-peptides

Anti-inflammatory natural products from vertebrate muscle



Anserine



Carnosine

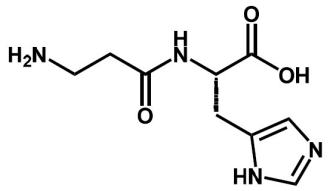
Chicken (100g):
Anserine (0.6g)
Carnosine (0.2g)

Salmon (100g):
Anserine (0.5g)

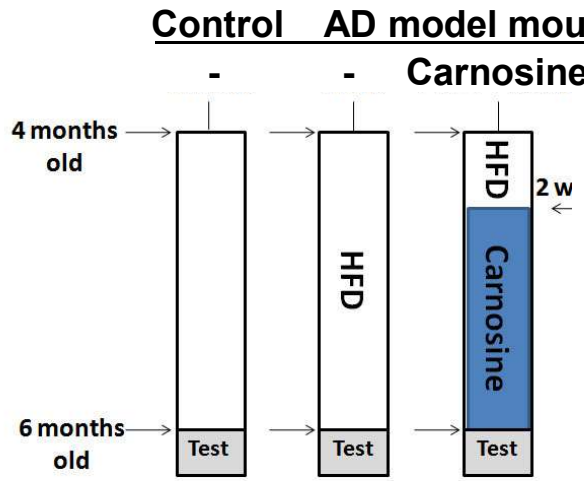
Ref. Boldyrev et al, Physiol. Rev. (2013)

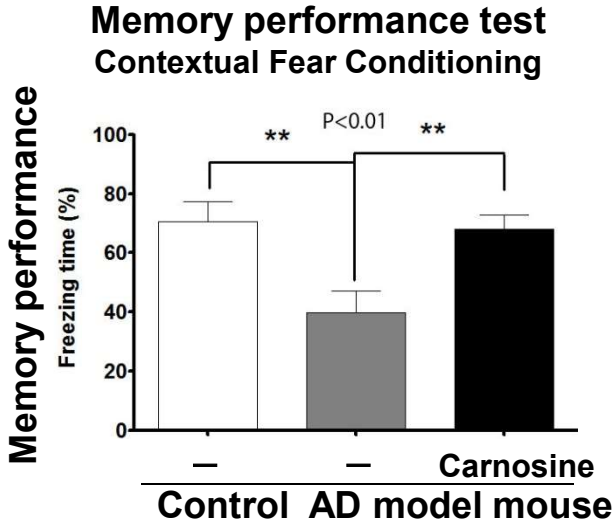
Carnosine protects neurovascular damage and memory decline in High Fat Diet-AD model mouse

Imidazoledipeptide
Carnosine
(β-alanyl-histidine)

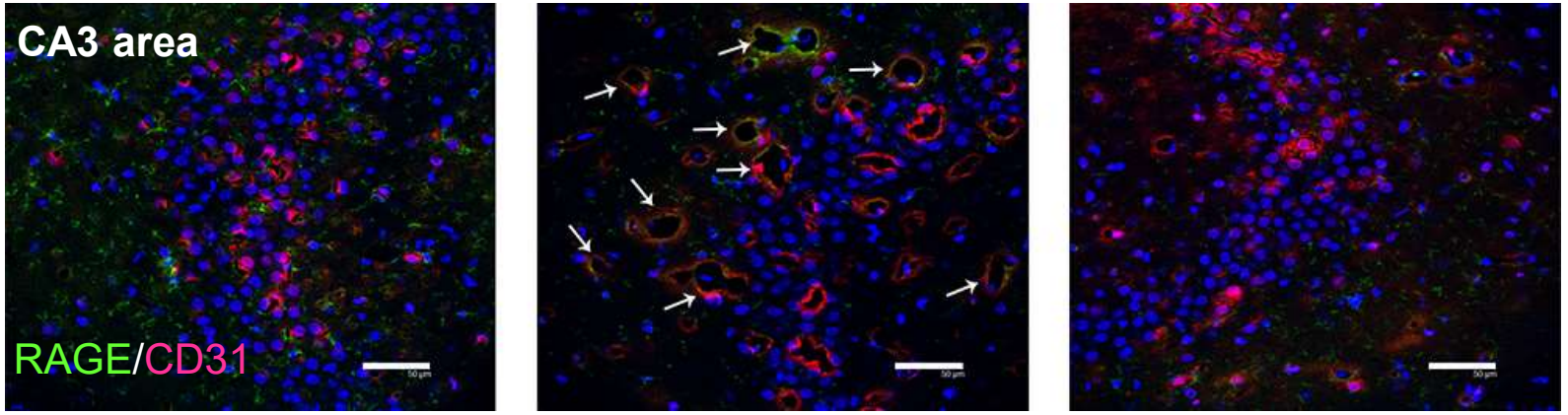


Sigma C9625





Neurovascular damage



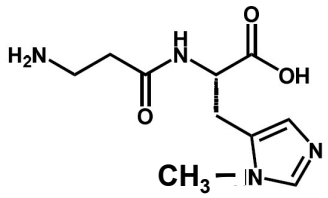
—
—
Carnosine

Control | **Alzheimer's Disease (AD) model mouse**

Herculano et al. *J Alzheimer`s Disease 33: 983-997 (2013)*

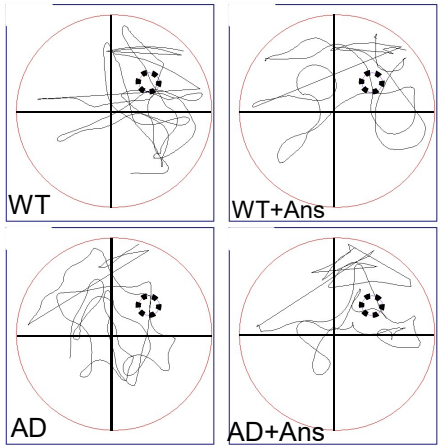
Anserine protects neuroinflammation and memory decline in 18 months old AD model mouse

Imidazoledipeptide
Anserine (Ans)
(β-alanyl-methyl histidine)

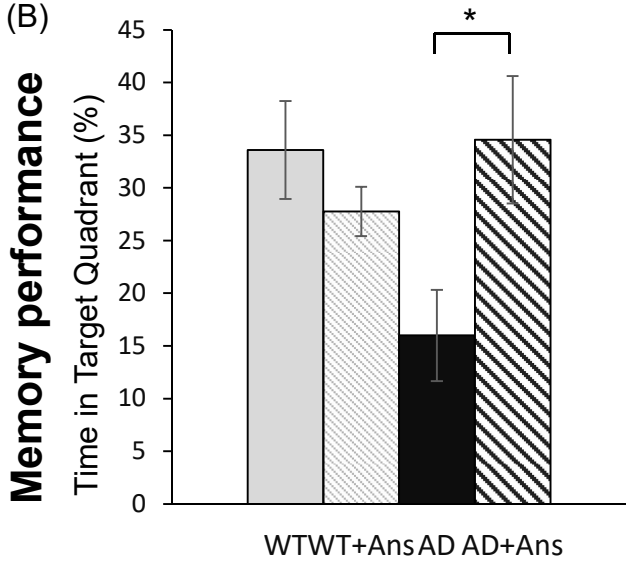


Purification
(>93 %) from Salmon

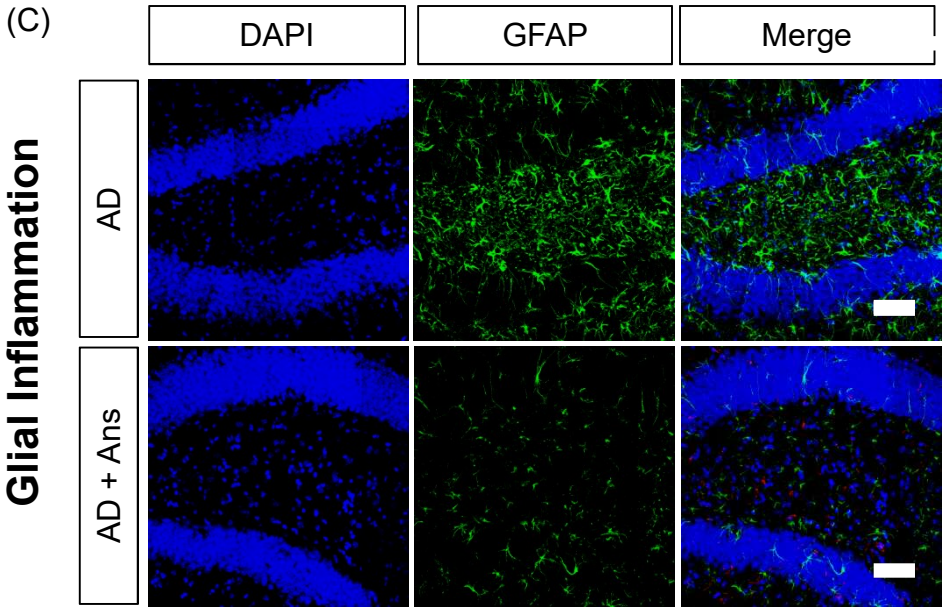
(A)



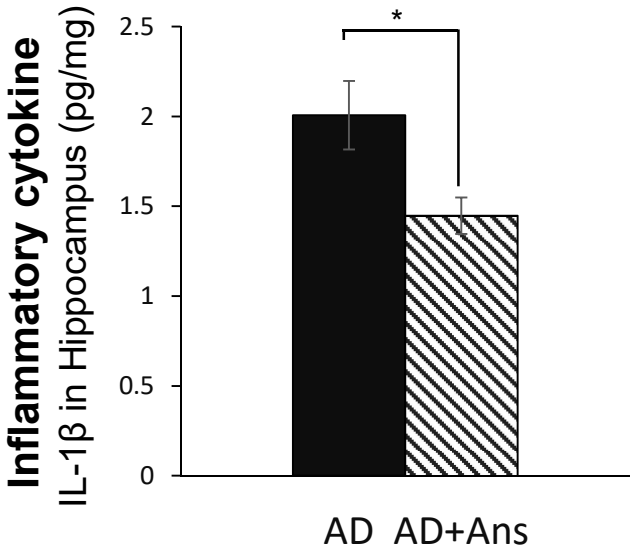
(B)



(C)

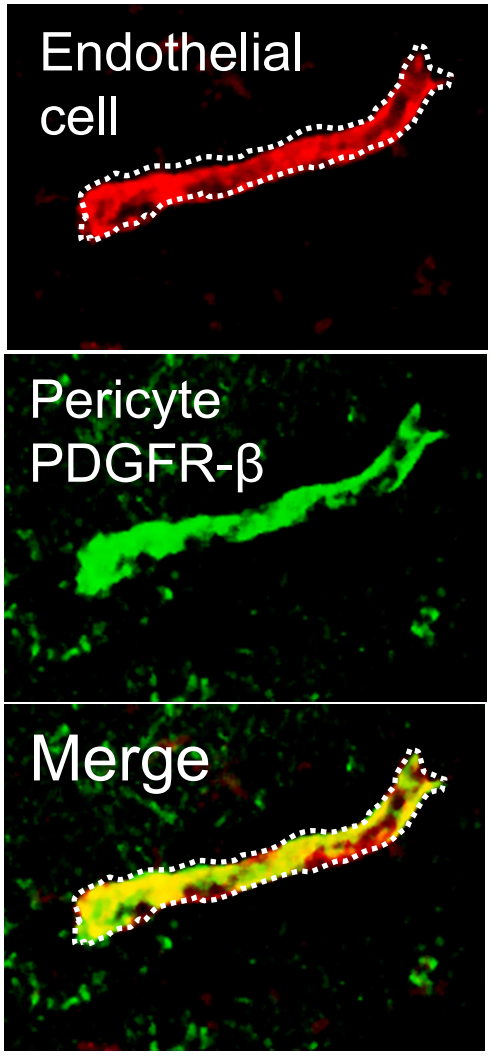


(D)

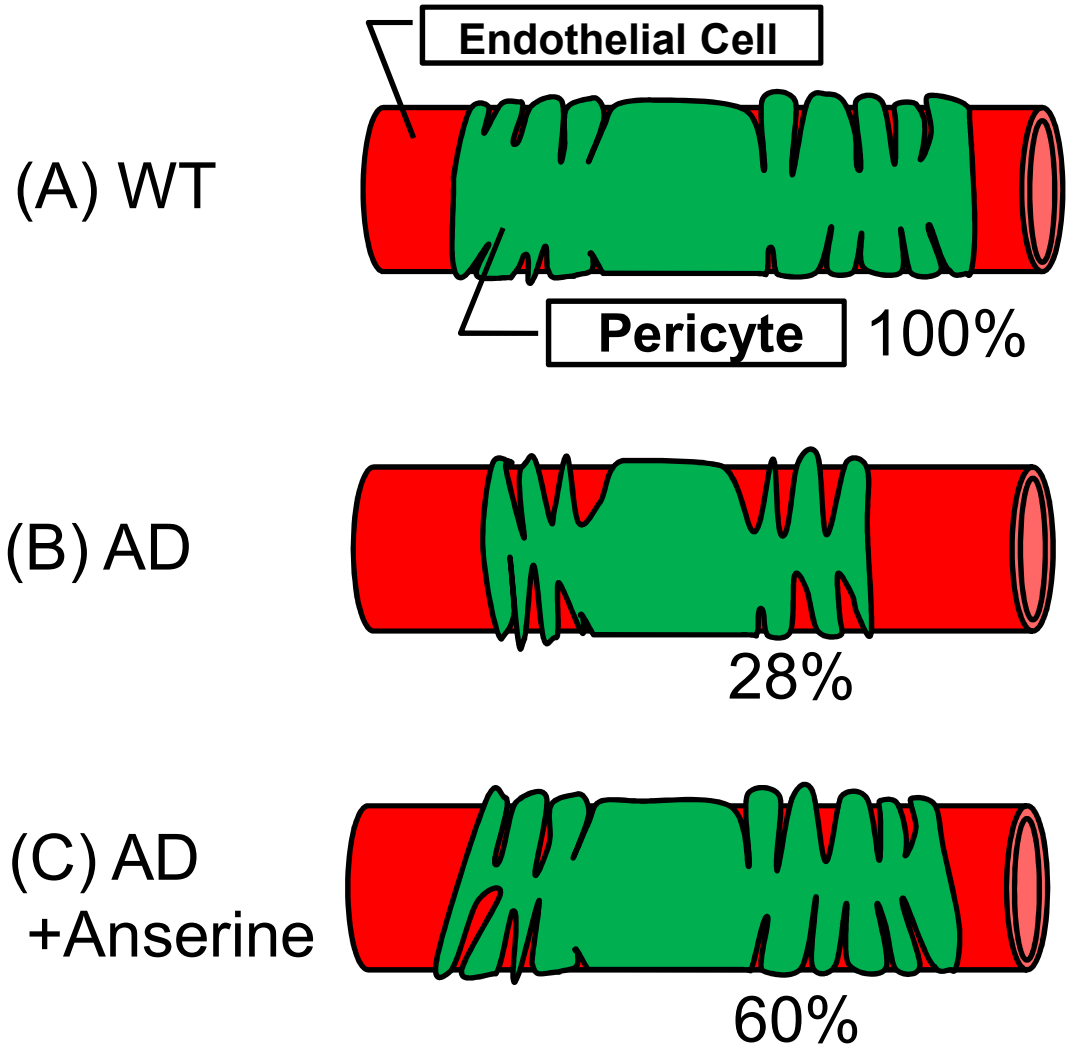


Kaneko et al. *Scientific Reports (2017)*

Anserine recovers degeneration of brain capillary pericytes



Pericyte staining with anti-PDGFR-β



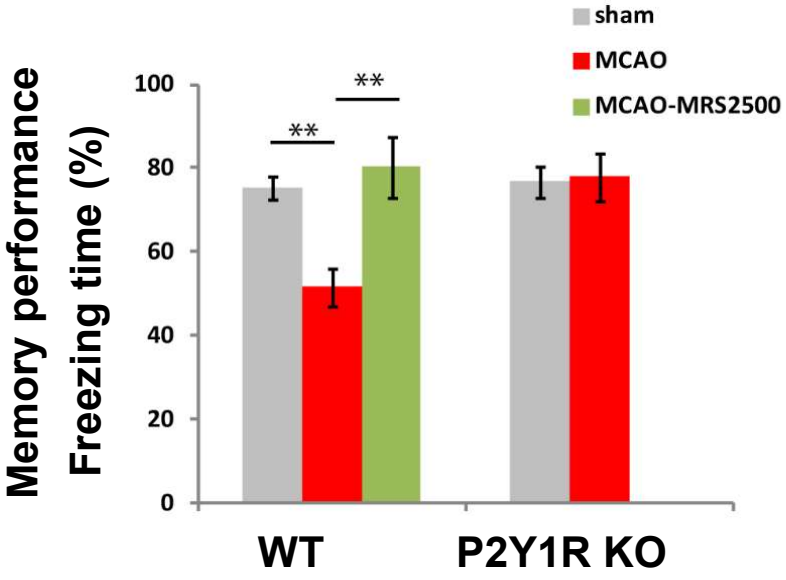
Kaneko et al. *Scientific Reports* (2017)

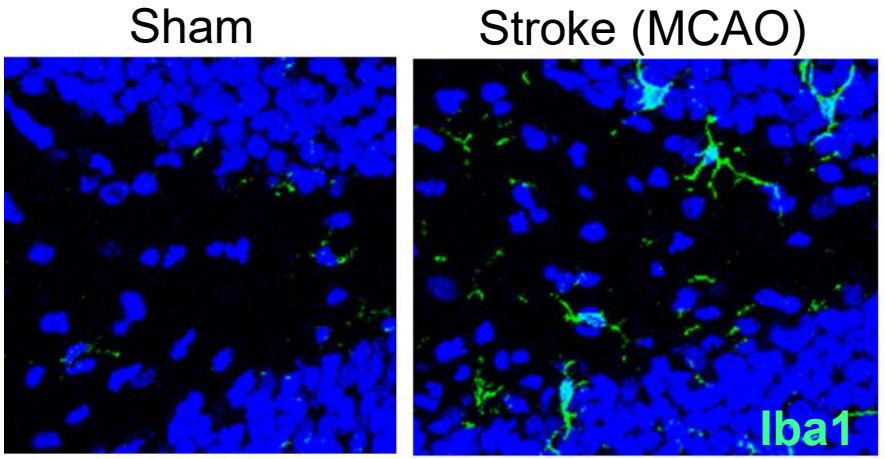
Aging and Anti-Aging of Cognitive Functions in Mouse

1) Alzheimer's Disease model mouse showed declines in cognitive function and glial neuroinflammation.

2) The control of neuroinflammation in AD model mouse leaded to the improvement of cognitive functions.

3) Cognitive declines in Stroke model mouse were also prevented in (less glial neuroinflammation) P2Y1R knock-out mouse.





Chin et al. *J Neuroinflammation* (2013)

Aging and Anti-Aging of Cognitive Functions

- Translation from Animal Models to Human clinical trials -

Species	Mouse	Human
Behavior Tests	Morris Water Maze	Wechsler Memory Scale
	Contextual Fear Conditioning	Clinical Dementia Rating (CDR)
Brain Examinations	Brain Tissue Staining	Brain MRIs VBM, fMRI ASL, DTI

Imidazoledipeptide ameliorates memory decline in elderly people

Elderly People (60-80 years old) | **Random Grouping (Active:Placebo=1:1)** | **Test Schedule (pre and post tests)** | **Statistics** Two-way repeated ANOVA

Active
Imidazoledipeptide
Carnosine 0.25 g/d
Anserine 0.75 g/d

6 month
BL FU

BL: Base Line, FU: Follow Up

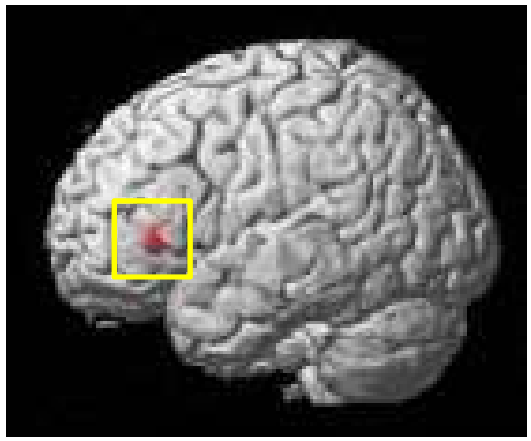
Active Group

Placebo
(Test food from Nipponham)

6 month
BL FU

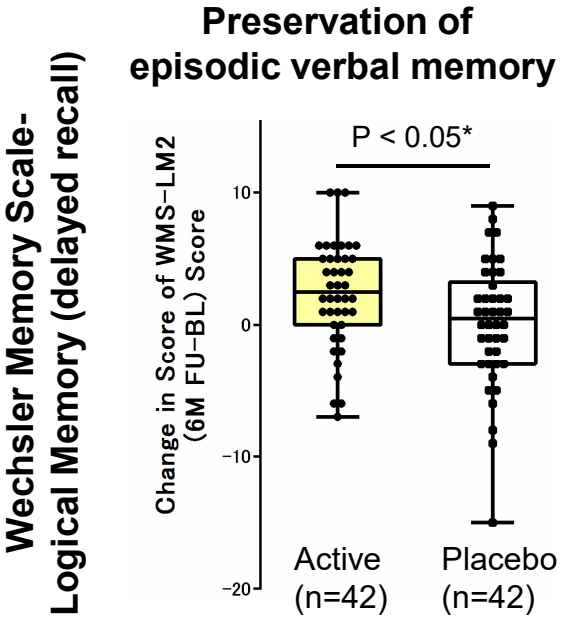
Placebo Group

Preservation of prefrontal cortex



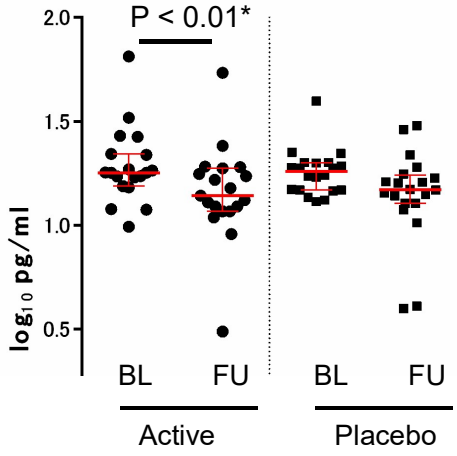
Active vs Placebo
VBM analysis ($p < 0.005$)

Preservation of episodic verbal memory



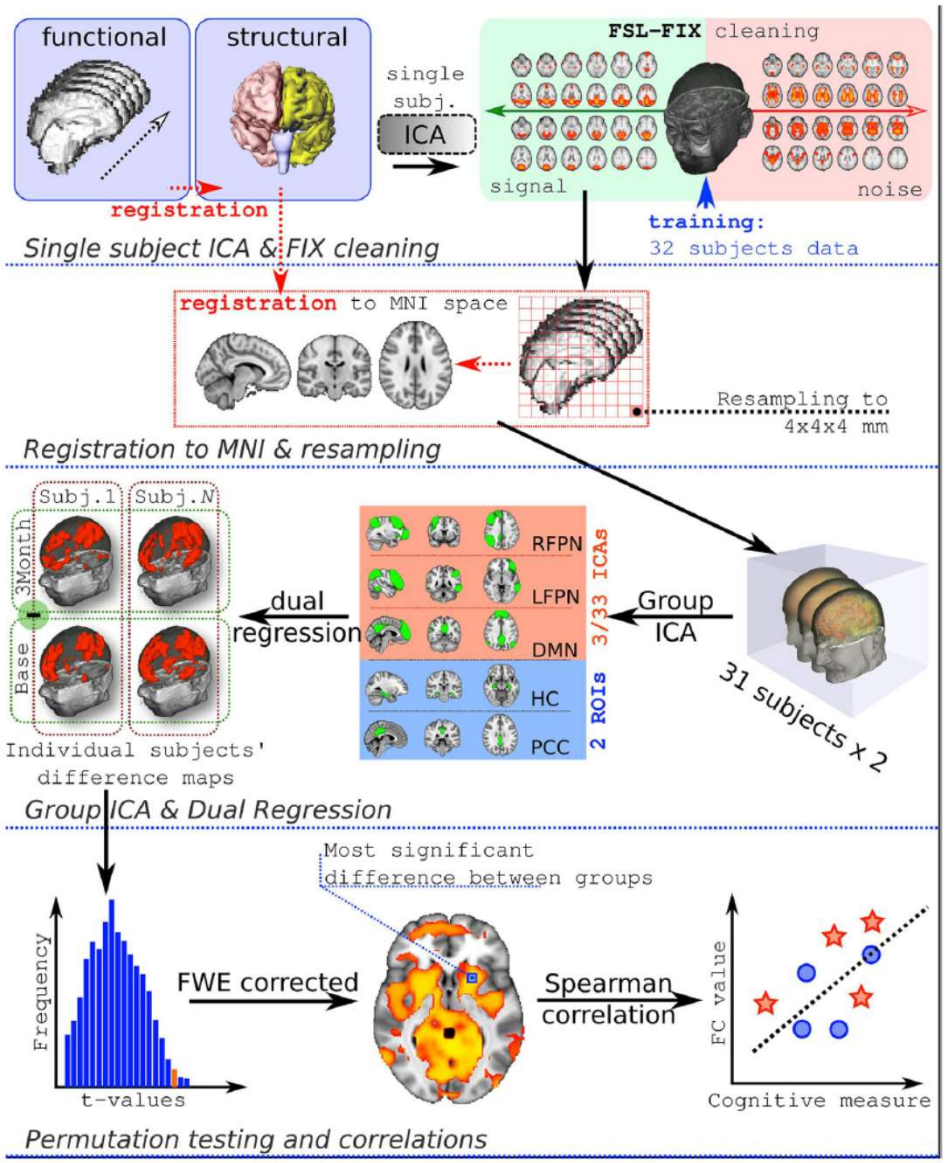
BL: Base Line, FU: Follow Up

Decrease in Inflammatory cytokine, IL-8, in plasma

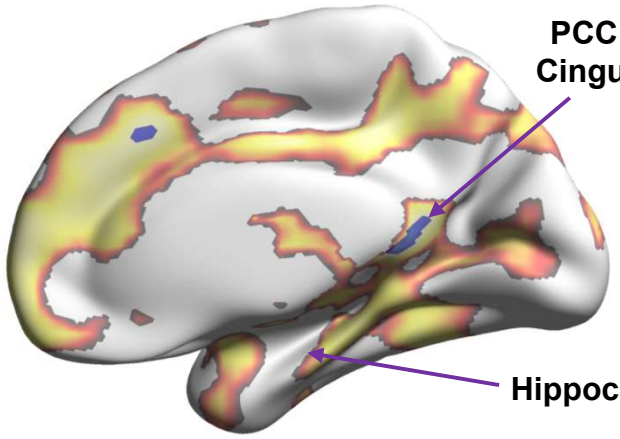


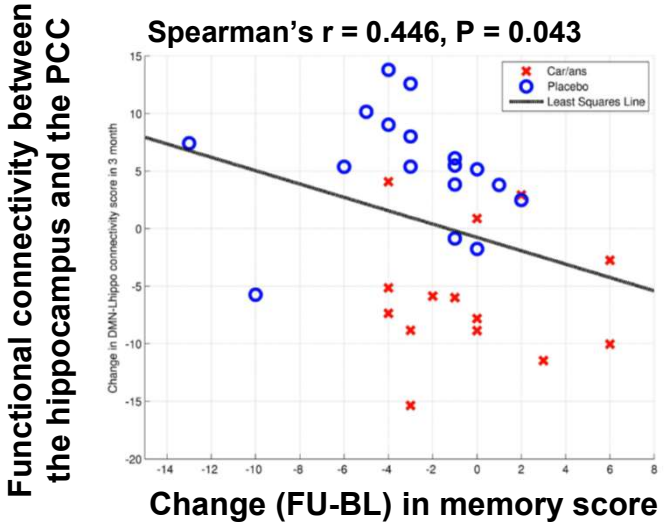
Hisatsune et al. *J Alzheimer Dis* (2016)

Alteration in network activity (fMRI study)



Resting-state fMRI analysis

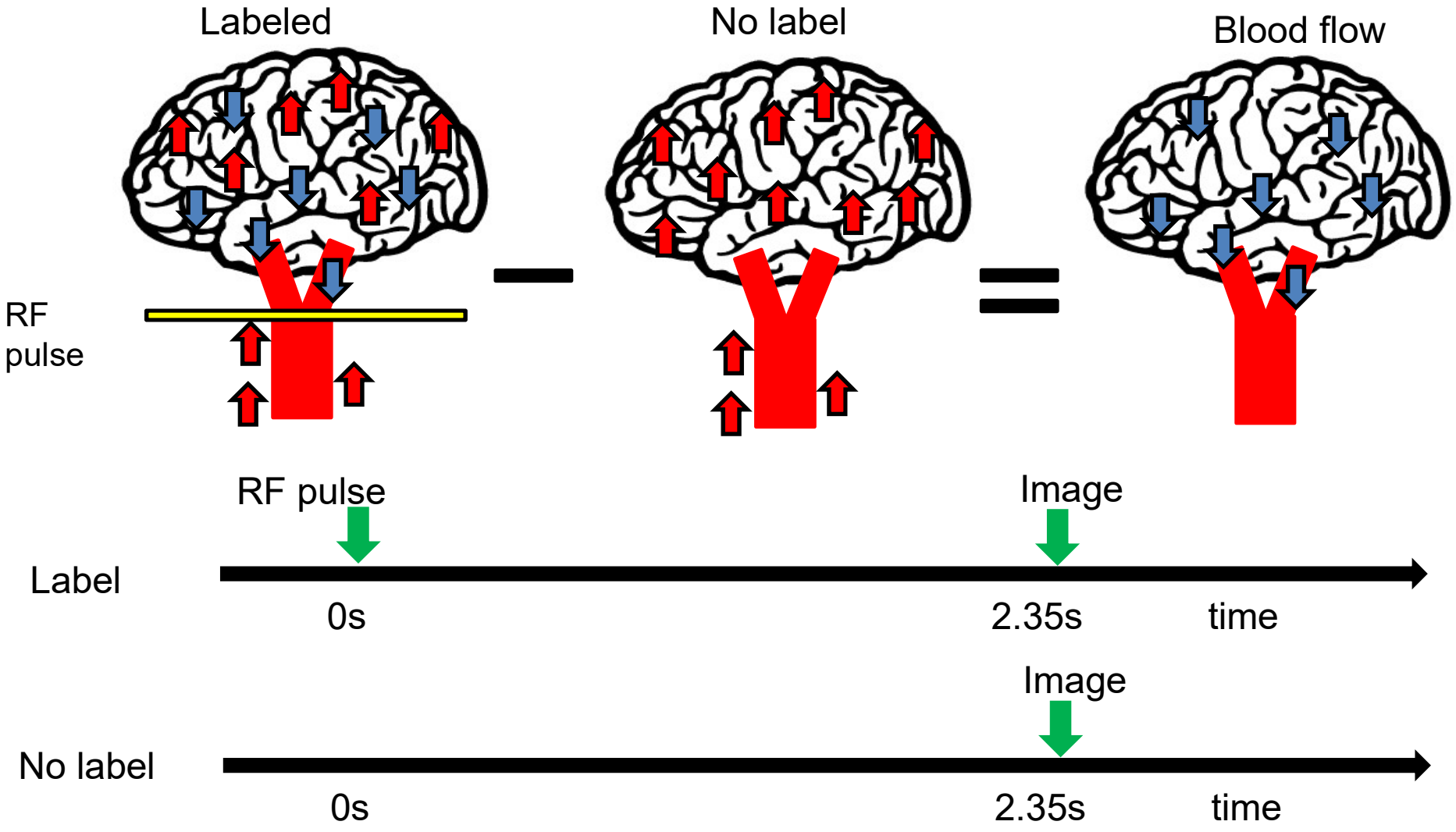




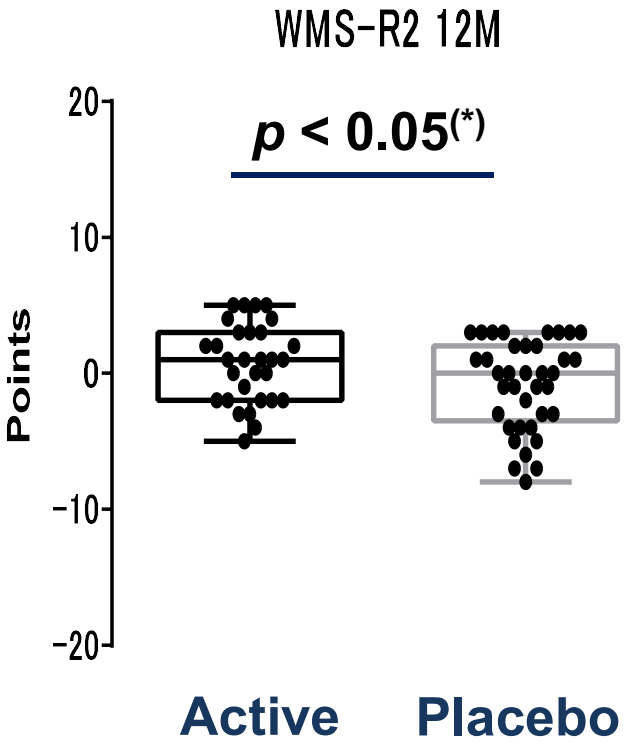
Rokicki et al. *Frontier Aging Neuroscience* (2015)

In collaboration with NCNP, IBIC (Dr. Matsuda)

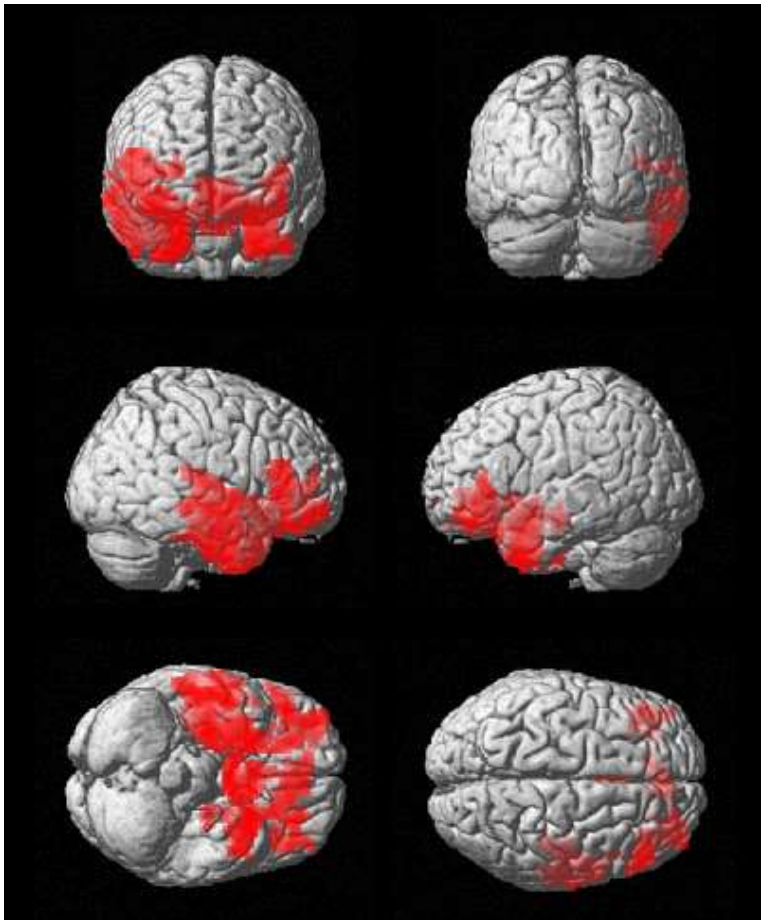
Arterial Spin Labeling (ASL): to evaluate brain blood flow



Effect of ACS on the preservation of brain blood flow at the frontal areas



Ding et al. *Aging and Disease* (2017)



Brain Areas of Blood flow preservation

ApoE4 as a risk gene for AD

Japanese (2313)

E3/E3 71.8 %

E4/E3: 18.6 %

E4/E4: 1.9 %

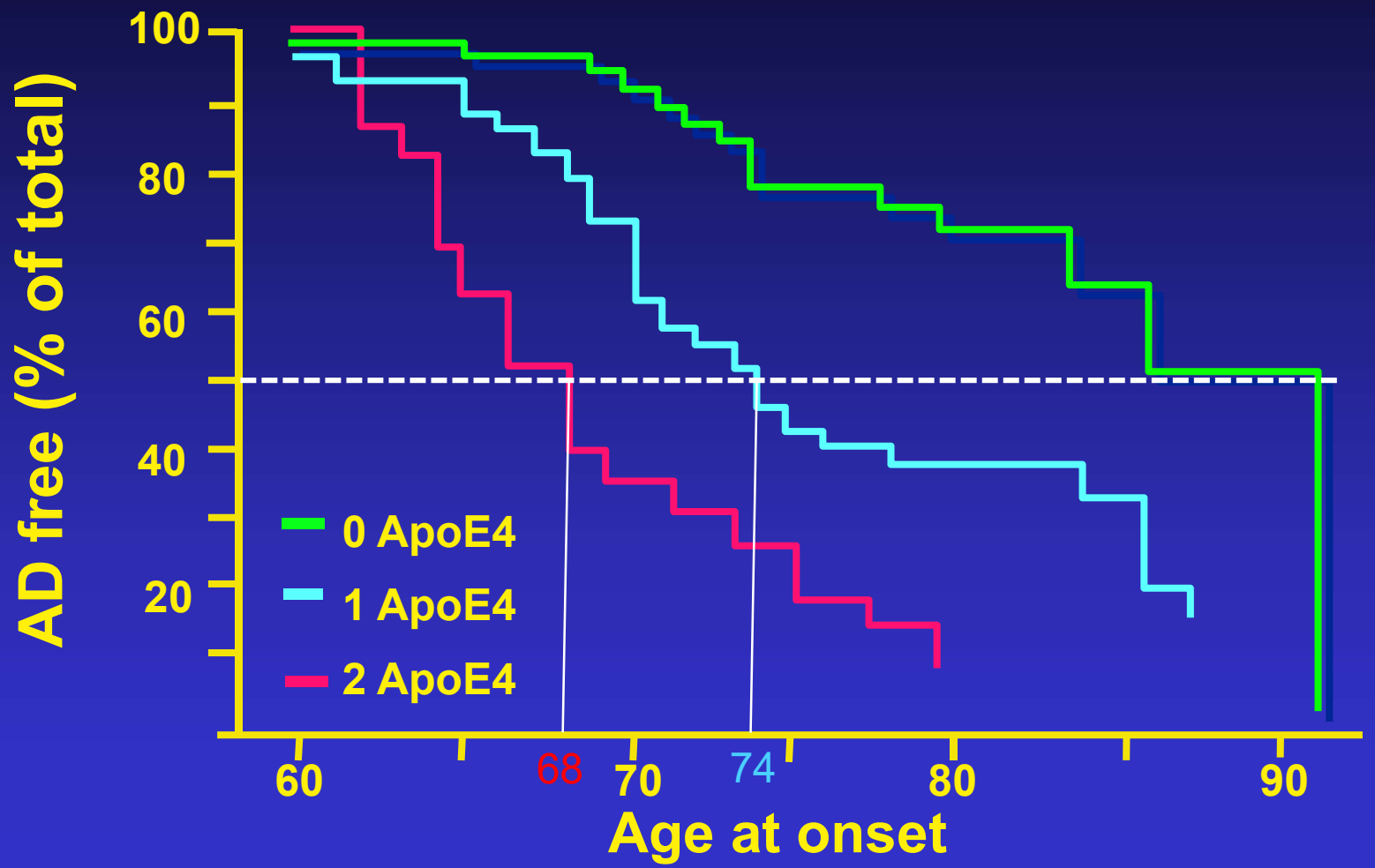
Table 3

APOE Genotype	No.	Odds Ratio (95% Confidence Interval)	Breslow-Day *P* Value†
		Japanese	
ε3/ε3	1661	1.0 (Referent)	. . .
ε2/ε2	9	1.1 (0.1-17.2)	.52
ε2/ε3	149	0.9 (0.4-2.5)	.84
ε2/ε4	19	2.4 (0.4-15.4)	.80
ε3/ε4	430	5.6 (3.9-8.0)	.11
ε4/ε4	45	33.1 (13.6-80.5)	.62

*Odds ratios for *APOE* genotypes derived assuming a reference odds ratio of 1 for *APOE* ε3/ε3 genotype.
†These *P* values are a test for heterogeneity of odds ratios for genotype among data sets.

Lindsay et al., JAMA 278(16):1349-1356 (1997)

Acceleration AD in ApoE4 carrier



Corder et al. *Science* (1993)

Aging and Disease
www.aginganddisease.org
Volume 9, Number 3; xxx-xx, June 2018
http://dx.doi.org/10.14336/AD.2017.0809

Original Article

Anserine/Carnosine Supplementation Preserves Blood Flow in the Prefrontal Brain of Elderly People Carrying APOE e4

Qiong Ding[1], Kitora Tanigawa[1], Jun Kaneko[1], Mamoru Totsuka[2], Yoshinori Katakura[3], Etsuko Imabayashi[4], Hiroshi Matsuda[4], Tatsuhiro Hisatsune[1*]

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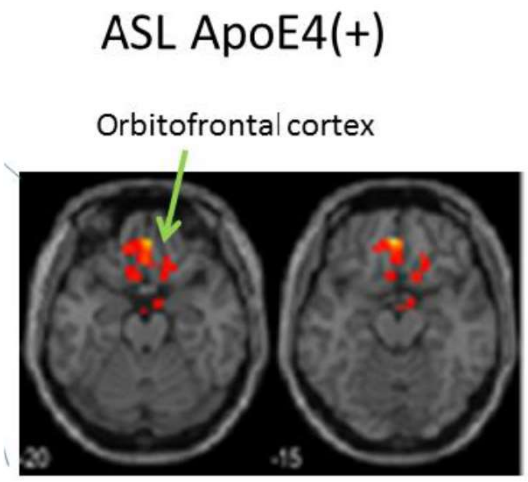
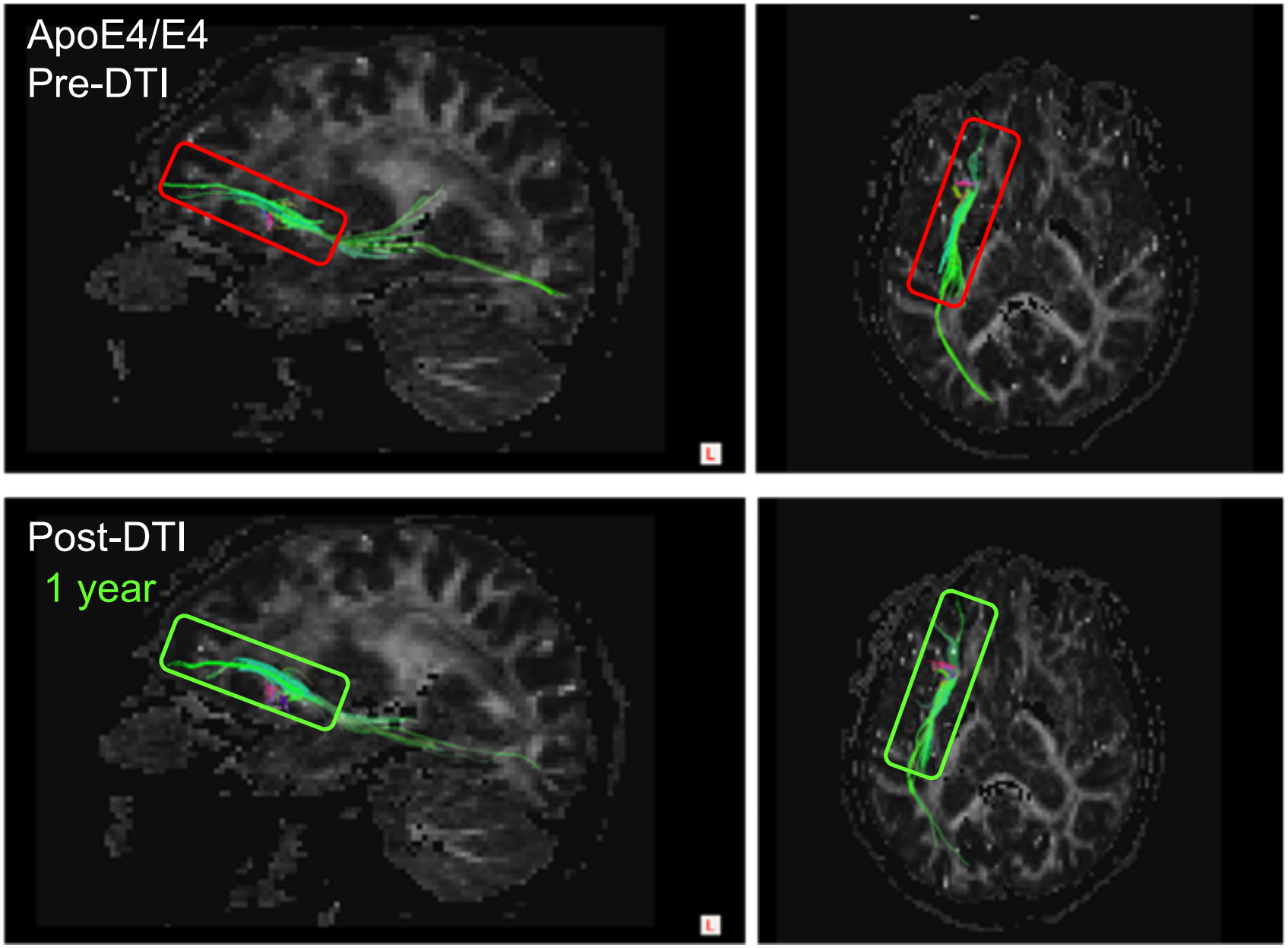
[2]Department of Applied Biochemistry, Graduate School of Agriculture and Life Sciences, The University of Tokyo,
[3]Graduate School of Systems Life Sciences, Kyushu University, Higashi-ku, Fukuoka, Japan
[4]Integrative Brain Imaging Center (IBIC), National Center of Neurology and Psychiatry, Tokyo, Japan

	APOE4$^+$ group			APOE4$^-$ group		
	E4/E4	E4/E3	E4/E2	E3/E3	E3/E2	E2/E2
Active	1	7	0	23	0	0
Placebo	2	2	0	33	0	0



Effect of ACS on the preservation of the prefrontal tract in an ApoE4 double carrier



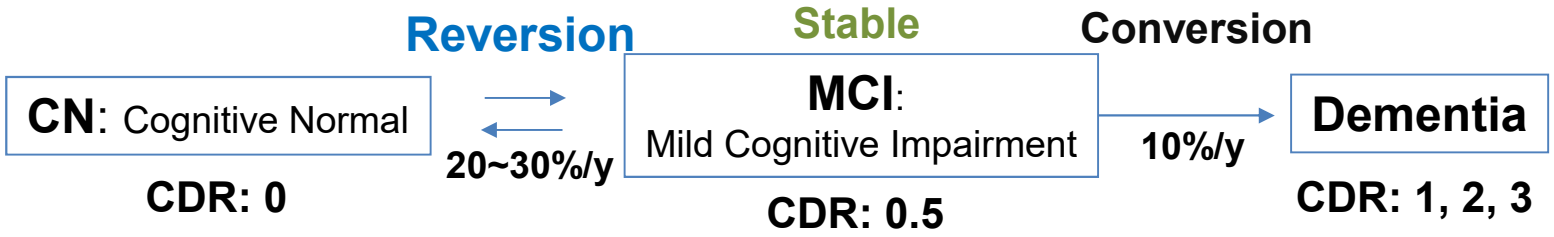
Randomized Controlled Trial to assess the effect of Anserine/Carnosine on the prevention of Alzheimer's Disease

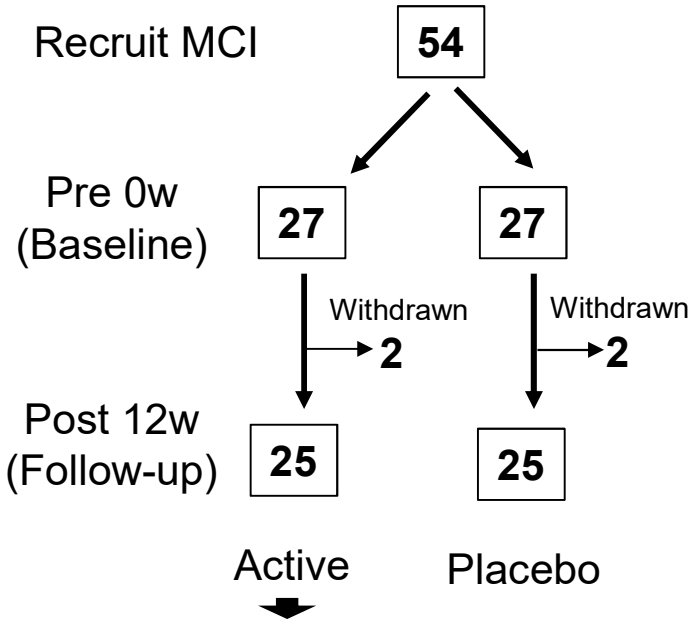
Participants: Mild Cognitive Impairment (MCI)

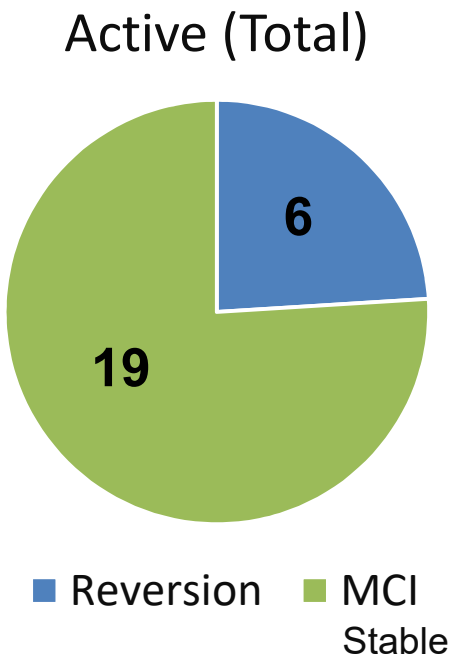
Visit	1	2	3	4	5	6
	Recruit	0 week Baseline	4 week	8 week	12 week Follow-up	Post observation
Informed Consent	○	◎				
Intervention Period						
Medical Examination	○	Anserine/Carnosine (1 g) / day			○	○
CDR (Clinical Dementia Rating) MMSE, WMS-LM2 ADAS-cog, GDS-S-J		○			○	
Blood Test		○			○	
Genotyping of ApoE		○				

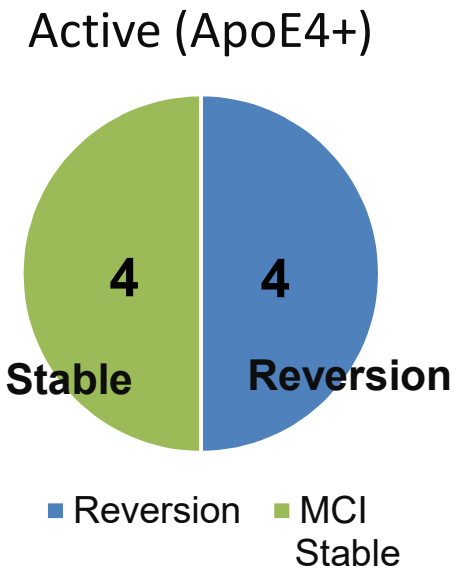
Under UMIN# R000023455

Interventional Clinical Study (individuals with MCI)









Masuoka et al. Presentation at Neuroscience 2017, Masuoka et al. (in revision)

CLINICAL DEMENTIA RATING (CDR):	0	0.5	1	2	3

	Impairment				
	None 0	**Questionable 0.5**	**Mild 1**	**Moderate 2**	**Severe 3**
Memory	No memory loss or Slight inconsistent forgetfulness	Consistent slight forgetfulness; Partial recollection of events; "benign" forgetfulness	Moderate memory loss; more marked for recent events; defect interferes with everyday activities	Severe memory loss; Only highly learned material retained; new material rapidly lost	Severe memory loss; only fragments remain
Orientation					
Judgment & Problem Solving					
Community Affairs					
Home and Hobbies					
Personal Care					

Memory Questions for Informant (Excerpt): **70's Male (ApoE4/E3) BL, FU**

1. Does he/she have a problem with his/her memory or thinking? ☑ Yes ☐ No
1a. If yes, is this a consistent problem (as opposed to inconsistent)? ☐ Yes ☑ No
2. Can he/she recall recent events? ☑ Usually ☐ Sometimes ☐ Rarely
3. Can he/she remember a short list of items (shopping)? ☑ Usually ☐ Sometimes ☐ Rarely
4. Has there been some decline in memory during the past year? ☑ Yes ☐ No
5. Is his/her memory impaired to such a degree that it would have interfered with his/her activities of daily life a few years ago (or pre-retirement activities)? (collateral sources opinion) ☐ Yes ☑ No
6. Does he/she completely forget a major event (e.g., trip, party, family wedding) within a few weeks of the event? ☐ Usually ☑ Sometimes ☑ Rarely
7. Does he/she forget pertinent details of the major event? ☑ Usually ☑ Sometimes ☐ Rarely
8. Does he/she completely forget important information of the distant past (e.g., birthdate, wedding date, place of employment)? ☐ Usually ☐ Sometimes ☑ Rarely

http://alzheimer.wustl.edu/adrc2/Images/CDRWorksheet.PDF

Reports regarding reversion from MCI

Effect of reversion from mild cognitive impairment (MCI) on cumulative incidence of dementia

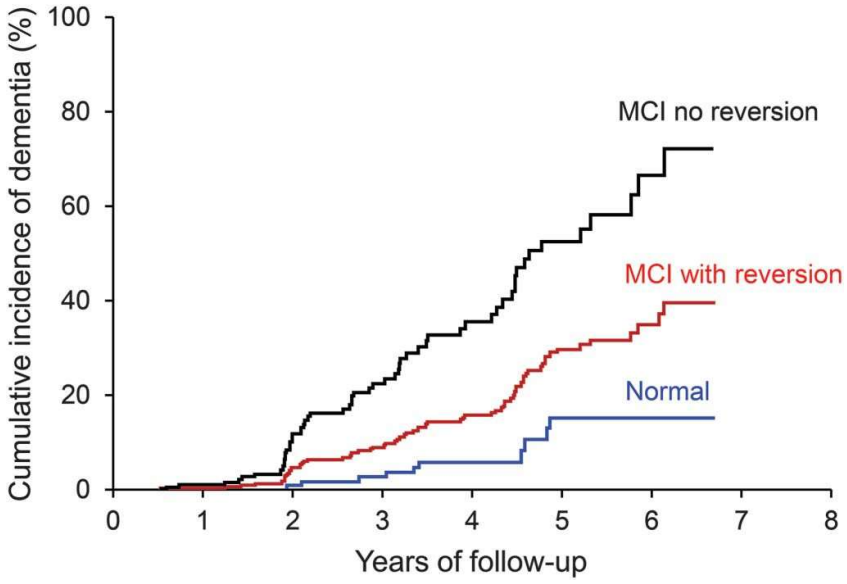


Figure 2 Cumulative incidence of dementia in subjects who developed MCI and did not revert to CN, subjects who developed MCI and reverted to CN, and subjects who were CN at baseline

Roberts et al. *Neurology* (2014)

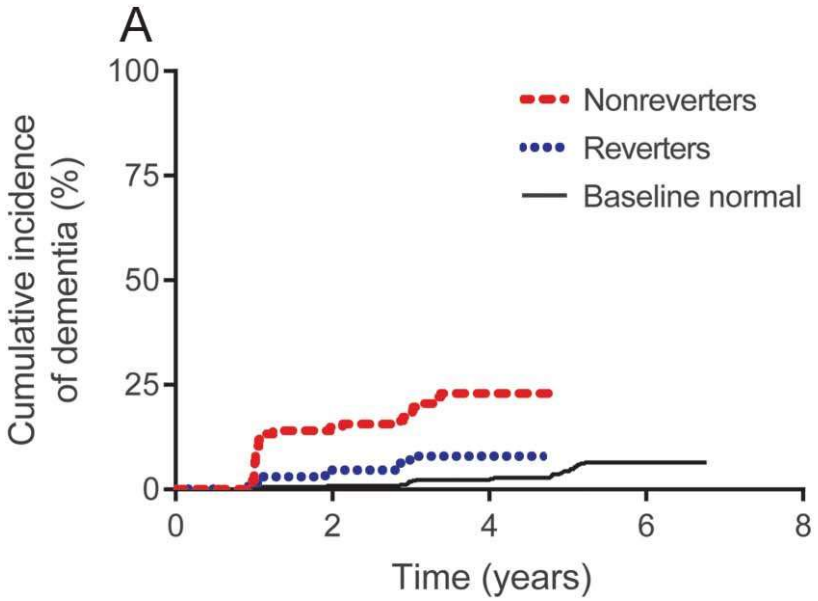


Figure 2 Effect of reversion from mild cognitive impairment (MCI) on cumulative incidence of dementia

Aerts et al. *Neurology* (2017)

Summary (Human Clinical Studies)

1) **Regenerative Medicine** to reduce the risk of Dementia can be feasible in Individuals with Mild Cognitive Impairment (MCI). MCI is a reversible transition stage between cognitive normal (CN) and Alzheimer's Disease (AD). Reversion, from MCI to CN, reduces the risk of the onset of AD, to about 50%.

2) **Neuroinflammation** as a downstream cellular responses is a target to develop regenerative medicine for Dementia.

3) **Anserine/Carnosine**, natural products from animal meat (imidazoledipeptides), suppressed neuroinflammation-related neurovascular dysfunction and cognitive declines in elders.

4) **In MCI Individuals carrying ApoE4, a major AD risk gene,** Anserine/Carnosine supplementation induced reversion and may reduce the risk of AD onset. Large scale clinical study to verify its effect is definitely awaited.

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