October/November, 2024

Invest in RIKEN BIO Co. Ltd.

Proposal for generating "RIKEN CBS-Industry Collaborative Center for Preclinical Alzheimer's Disease Research"

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RIKEN Center for Brain Science
JAPAN

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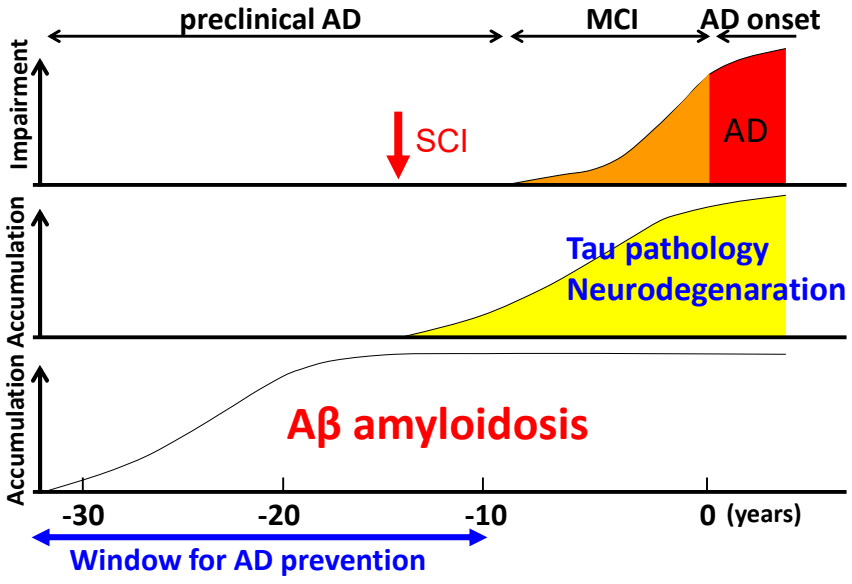
Pathological and neurological chronology leading to AD



AD is a disorder to prevent rather than to cure because neurons and circuits cannot regenerate after degeneration.

Let's make the world ADless by making preclinical AD treatable.

Prelude: Predicting the future in AD research 1.

We discovered that $A\beta_{3pE\text{-}X}$ is the major Aβ species that accumulates in human brain.

Saido, T.C., Iwatsubo, T., Mann, D.M., Shimada, H., Ihara, Y., and Kawashima, S. (1995). Dominant and differential deposition of distinct β-amyloid peptide species, $A\beta_{N3(pE)}$, in senile plaques. ***Neuron*** *14*, 457-466.

↓

Donanemab, humanized anti-$A\beta_{3pE\text{-}X}$ monoclonal antibody, was as effective as lecanemab in clinical trials and approved by US FDA.

Sims, J.R., Zimmer, J.A., Evans, C.D., Lu, M., Ardayfio, P., Sparks, J., Wessels, A.M., Shcherbinin, S., Wang, H., Monkul Nery, E.S., *et al.* (2023). Donanemab in Early Symptomatic Alzheimer Disease: The TRAILBLAZER-ALZ 2 Randomized Clinical Trial. ***JAMA*** *330*, 512-527.

Prelude: Predicting the future in AD research 2.

We predicted possible presence of genetic risk factor(s) in the *MME* gene that encodes neprilysin, a major Aβ-degrading enzyme.
Iwata, N., Tsubuki, S., Takaki, Y., Shirotani, K., Lu, B., Gerard, N.P., Gerard, C., Hama, E., Lee, H.J., and Saido, T.C. (**2001**). Metabolic regulation of brain Aβ by neprilysin. ***Science*** *292*, 1550-1552.

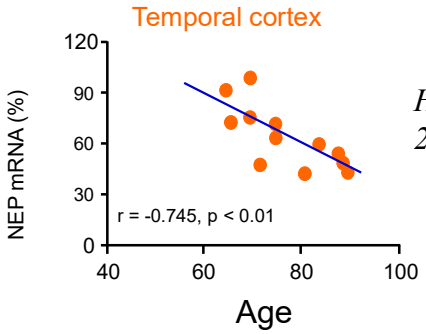
↓

A recent GWAS identified risk SNPs/mutations in the *MME* gene.
Bellenguez, C., Küçükali, F., Jansen, I.E., Kleineidam, L., Moreno-Grau, S., Amin, N., Naj, A.C., Campos-Martin, R., Grenier-Boley, B., Andrade, V., *et al.* (**2022**). New insights into the genetic etiology of Alzheimer's disease and related dementias. ***Nat Genet*** *54*, 412-436.

↓

The exonic mutation, M8V, reduces neprilysin's action on Aβ metabolism.

Identification of the *MME* mutation supports the role of aging-associated neprilysin reduction in SAD pathogenesis and endorses use of neprilysin activity for treatment of preclinical AD.

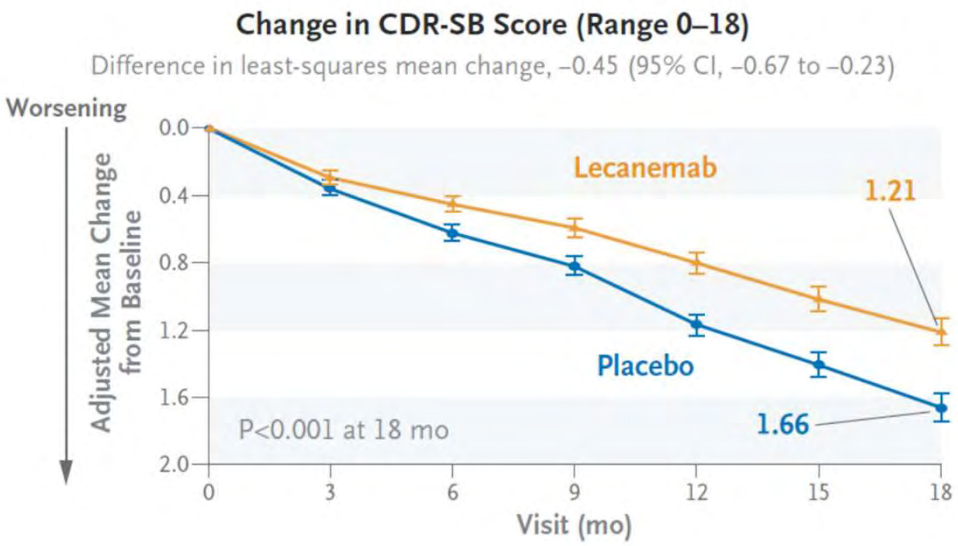


Hellström-Lindahl et al. Neurobiol. Aging 29, 210-221 (2008)

Therapeutic antibody, lecanemab, raised against Aβ oligomers

Lars Lannfelt (University of Upsala) generated a monoclonal antibody to soluble Aβ oligomers.

Lacanemab reduces a cognitive decline of early AD patients by 27%.

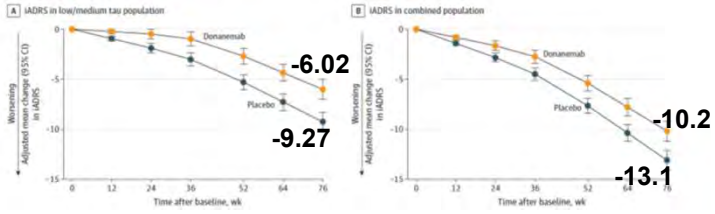


Another successful therapeutic antibody : donanemab (anti-Aβ_{3pE})

JAMA | Original Investigation

Donanemab in Early Symptomatic Alzheimer Disease
The TRAILBLAZER-ALZ 2 Randomized Clinical Trial

John R. Sims, MD; Jennifer A. Zimmer, MD; Cynthia D. Evans, PhD; Ming Lu, MD, MS, MPH; Paul Ardayfio, PhD; JonDavid Sparks, PhD; Alette M. Wessels, PhD; Sergey Shcherbinin, PhD; Hong Wang, PhD; Emel Serap Monkul Nery, MD; Emily C. Collins, PhD; Paul Solomon, PhD; Stephen Salloway, MD; Liana G. Apostolova, MD; Oskar Hansson, MD, PhD; Craig Ritchie, MD, PhD; Dawn A. Brooks, PhD; Mark Mintun, MD; Daniel M. Skovronsky, MD, PhD; for the TRAILBLAZER-ALZ 2 Investigators



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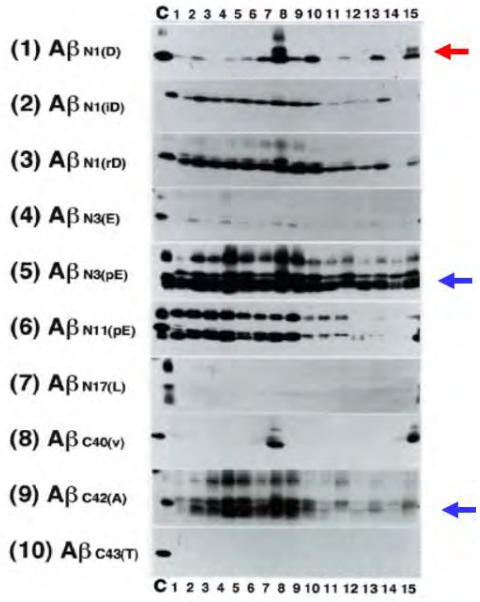
Aβ variants in AD brains.

Western blot analyses of brain extracts from AD patients. Samples provided by David MA Mann, University of Manchester.

Aβ3pE-42 is the predominant species.



Saido *et al.*, 1995; Saido, 2024; Iwata *et al.*, *Life Science Alliance*, in press.

Society for Neuroscience Meeting (2002)

Ron DeMattos (Lilly) generated the monoclonal antibody to Aβ3pE.

Modeling preclinical AD

Our strategy: Avoiding overexpression artifacts

1	Inserted cDNA(s) destroy endogenous genes.
2	APP is expressed in brain regions quite not identical to those of the wild-type brain.
3	Cell type-specific splicing of *APP* mRNA is lost.
4	Overexpressed APP and PSEN1 interact with cellular factors in an abnormal manner, i.e. APP with kinesin and JIP-1.
5	APP fragments possess physiological functions which can be perturbed by the overexpression. For instance APPs acts on a GABA receptor.
6	Overexpression of APP and PSEN1, membrane proteins, may cause artificial ER stress.
7	Calpain activation takes place in a manner independent of Aβ pathology. (Other calicium-dependent enzymes may also be mobilized.)
8	Aβ species different from those in AD brain accumulate.
9	Promoters present in the transgene(s) may perturb cellular trascription factor dynamics.
10	Secretase inhibitor(s) may not may not affect normal cleavage(s) due to the APP and PSEN1 mutations.
11	There may have been a phenotype bias in the selection of mouse lines.
12	Cross breeding with other mutants may cause even more complicated artifacts.
13	The geneme editing technology is not applicable.
14	Some lines show behavioral abnormality before any pathological changes. Some liens also die early.

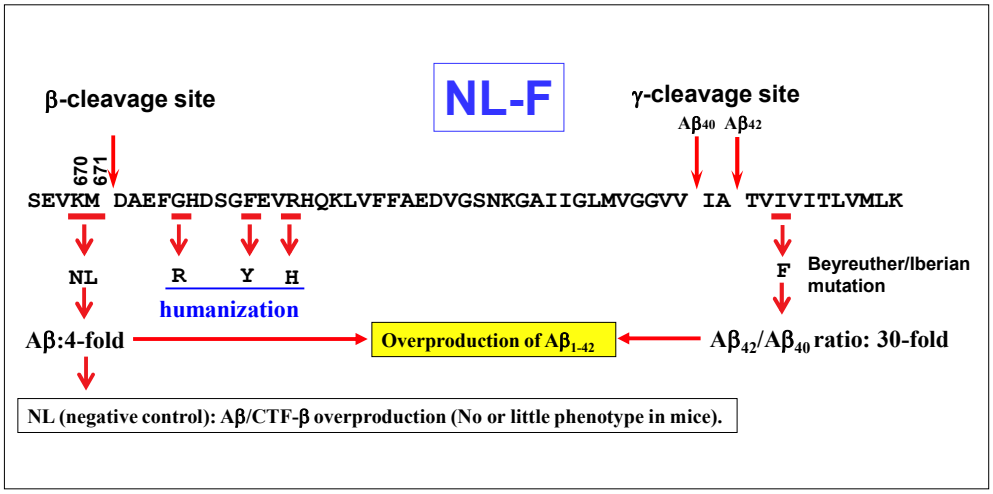
Alert!

Two uncertainties arise:

1. **Regarding why artifacts arise.**
2. **Regarding how artifacts affect interpretation of experimental results.**

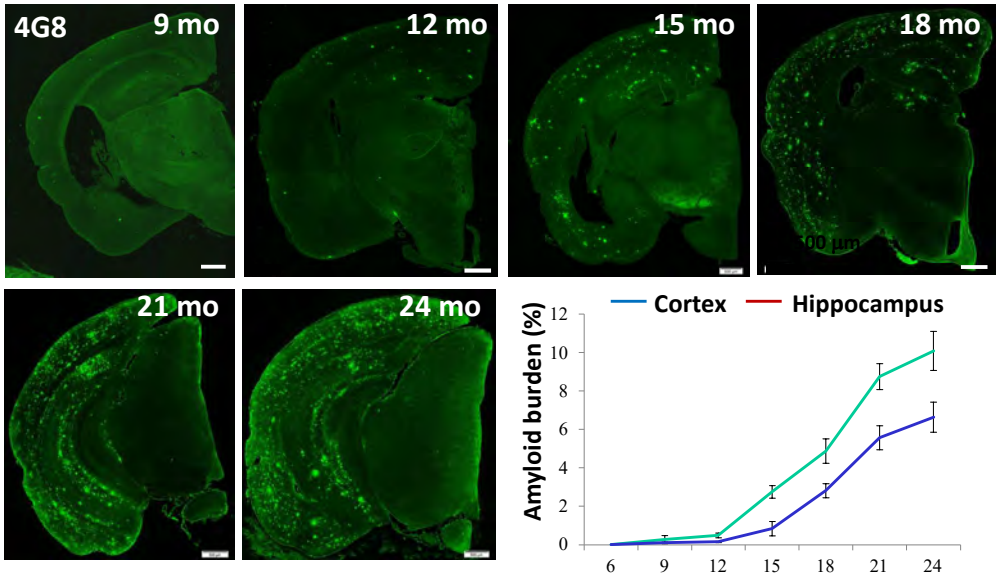
Saido (2024)

Game changer:
2nd generation knock-in mouse model of Aβ amyloidosis: *App^{NL-F}*



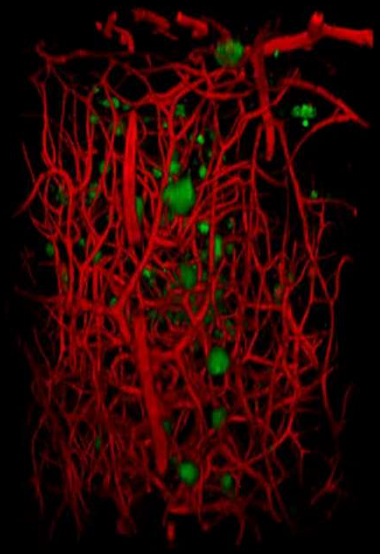
Saito *et al.* (*Nat Neurosci*, 2014)

Age-dependent Aβ deposition without APP overexpression



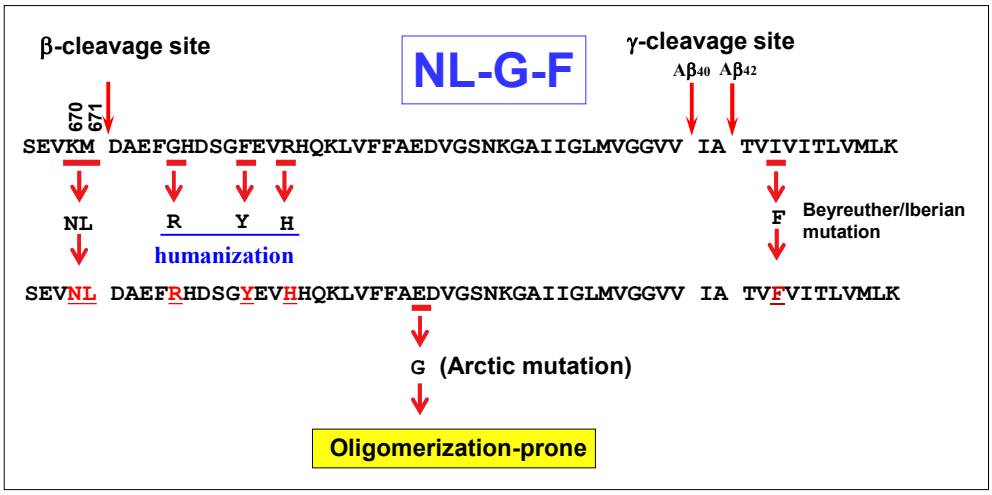
Aβ deposition was **detected from 6 months of NL-F/NL-F brains.**

3D imaging of Aβ pathology in the model mouse (animation)



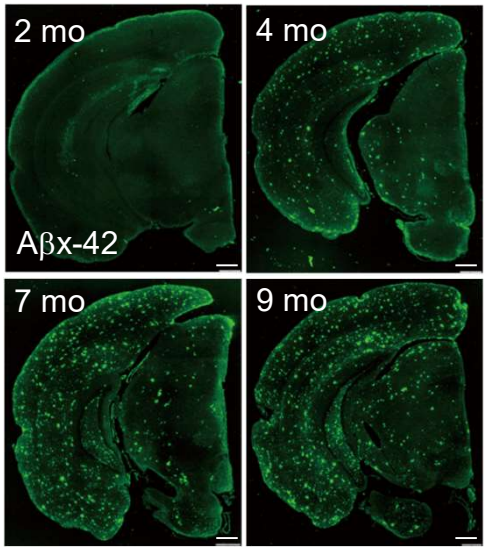
Red: blood vessels; Green: Aβ plaques Hama *et al. Nat Neurosci* (2015)

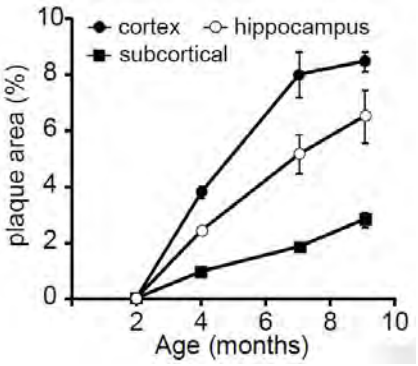
Another 2nd generation KI mouse model of Aβ amyloidosis: *App*$^{NL\text{-}G\text{-}F}$



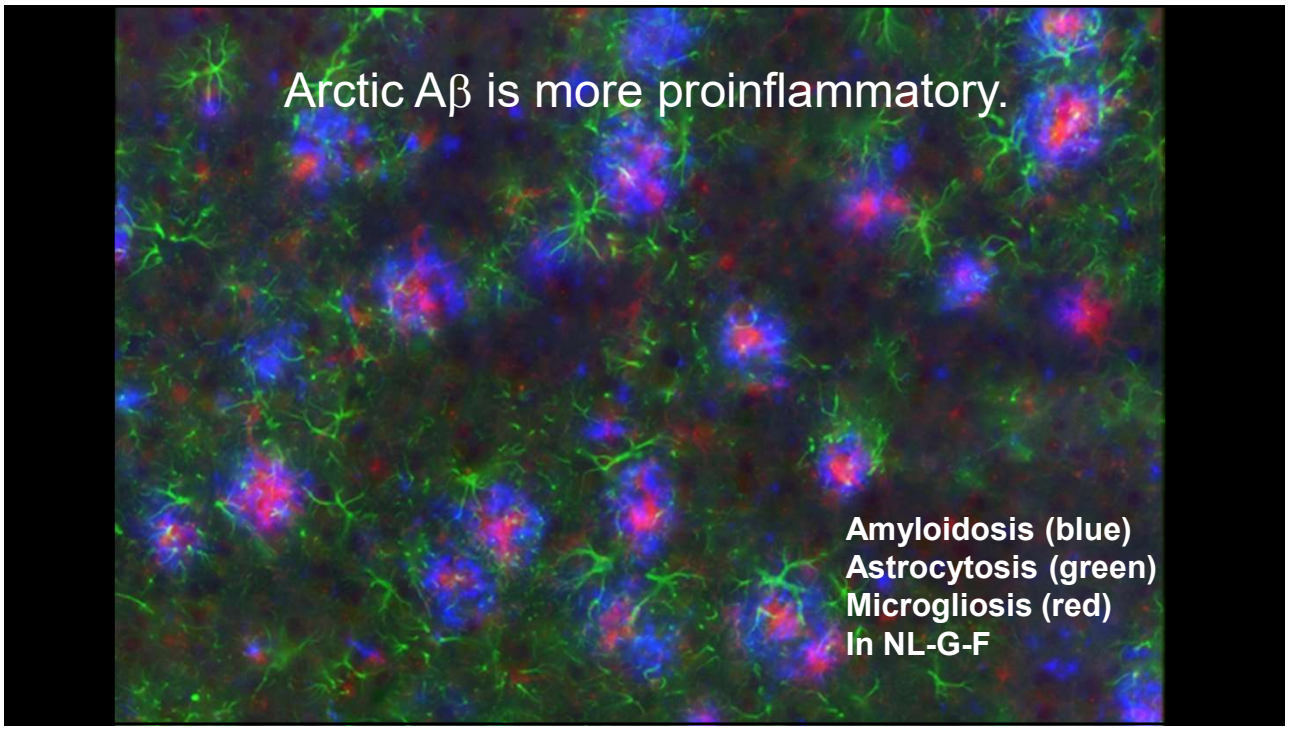
Saito *et al.* (Nat Neurosci, 2014)

Aβ42 pathology of NL-G-F KI mice





Aβ deposition appeared 3 times faster than **NL-F/NL-F** mice.



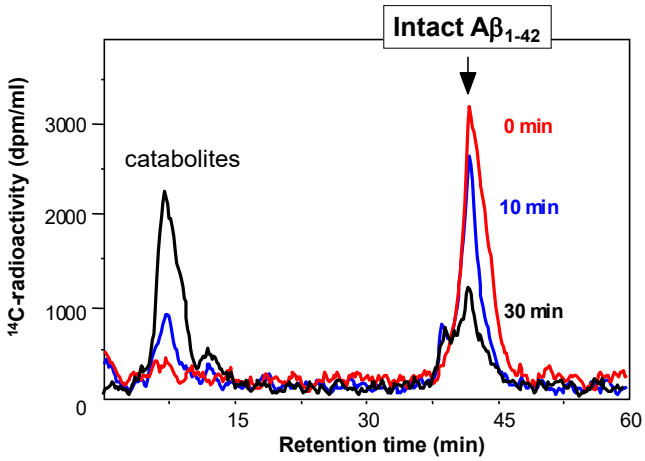
Single *App* knock-in mouse lines available.

1. hAβ: Negative control.
2. NL: Another negative control with increased CTF-β
3. NL-F: Accumulation of WT Aβ after 6 months
4. NL-G-F: Accumulation of Arctic Aβ after 2 months
5. G-F: Accumulation of Arctic Aβ after 4 months with normal β-secretase cleavage
6. NL-F x *Psen1*^{P117L}: Accumulation of WT Aβ after 2 months

Science without negative/positive control(s) is not good science.

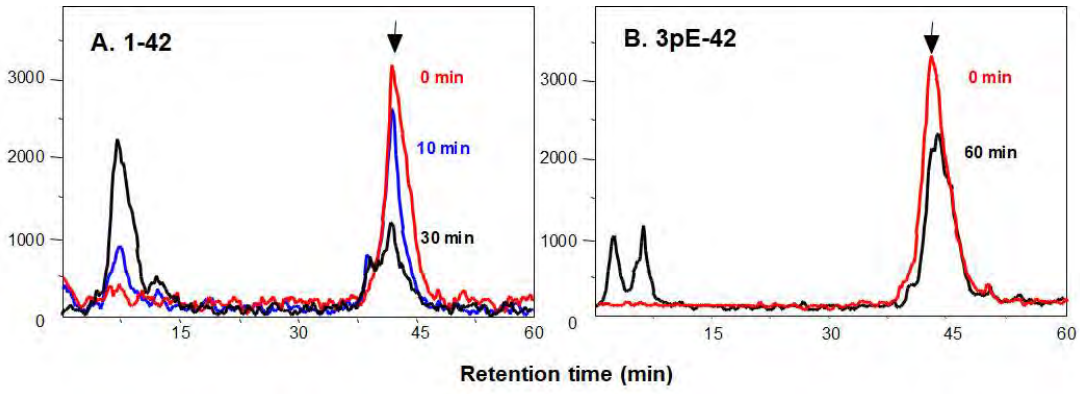
Catabolism of Aβ

Degradation of $A\beta_{1-42}$ in rat hippocampus



Iwata et al., *Nature Med.* (2000)

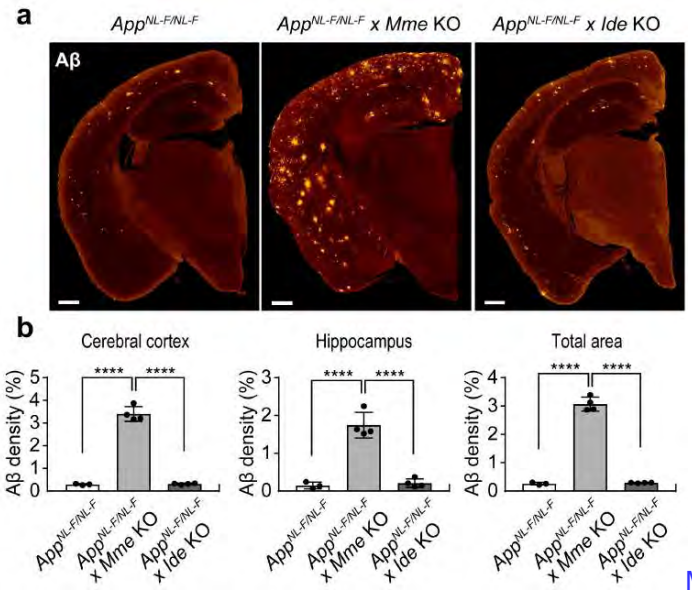
Degradation of $A\beta_{3pE-42}$ *in vivo*



Aβ3pE-42 is more resistant to *in vivo* catabolism.

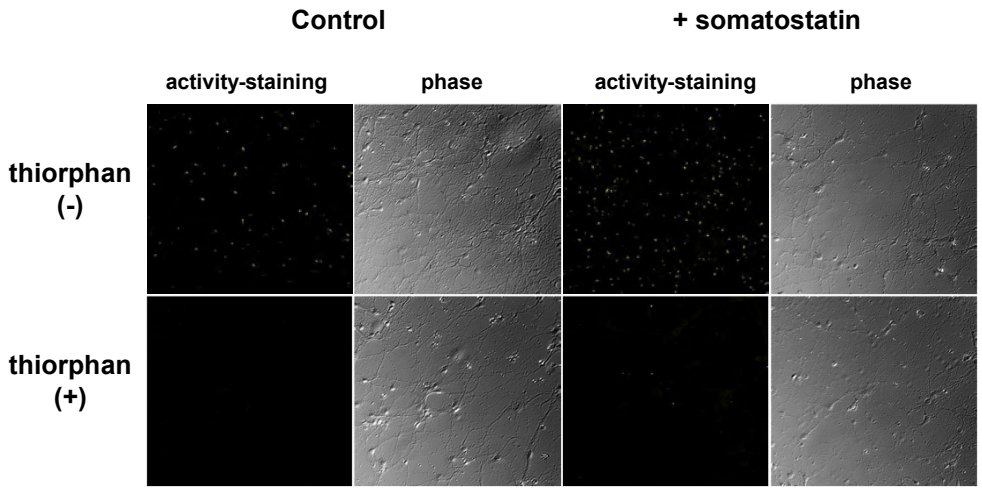
Iwata, *et al.*, *Life Science Alliance*, 2024.

Aβ depsition in *App*$^{NL-F}$ x *Mme* KO and *App*$^{NL-F}$ x *Ide* KO mice



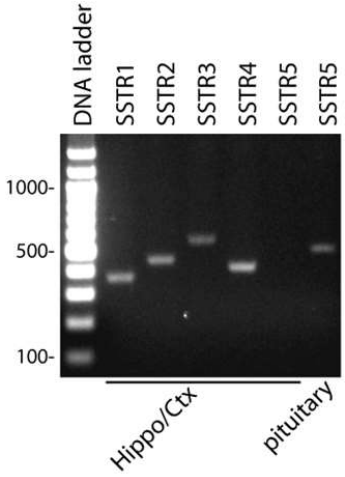
Morito *et al.* (submitted)

Somatostatin (SST) activates neuronal neprilysin.



Saito *et al.* (***Nat Med***, 2005)

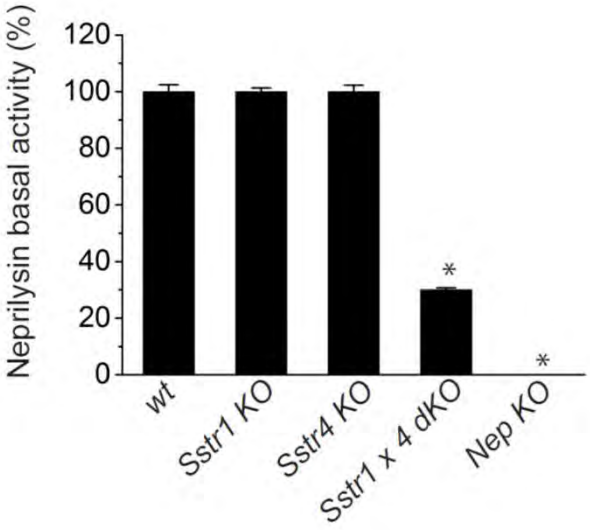
Expression of SSTR subtypes in the brain: SSTRs 1-4 are present in CNS.



Possibly, 4 homodimers & 6 heterodimers.

Nilsson *et al.*, under revision.

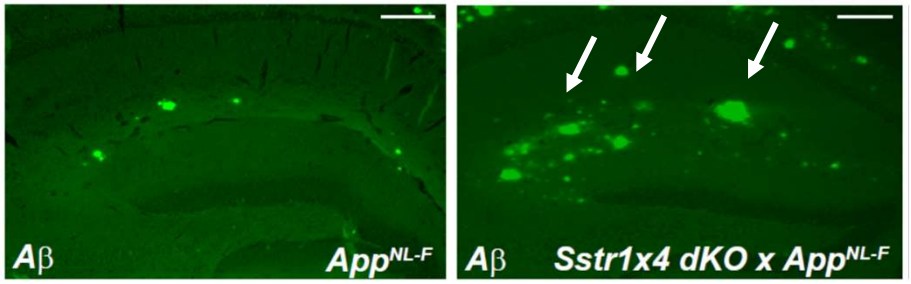
None of the <u>single</u> SSTR subtype-KO mouse-derived neurons showed any changes in neprilysin activity.



Combination of SSTR subtypes 1 & 4 regulates neprilysin activity *in vitro*.

Nilsson *et al.*, under revision.

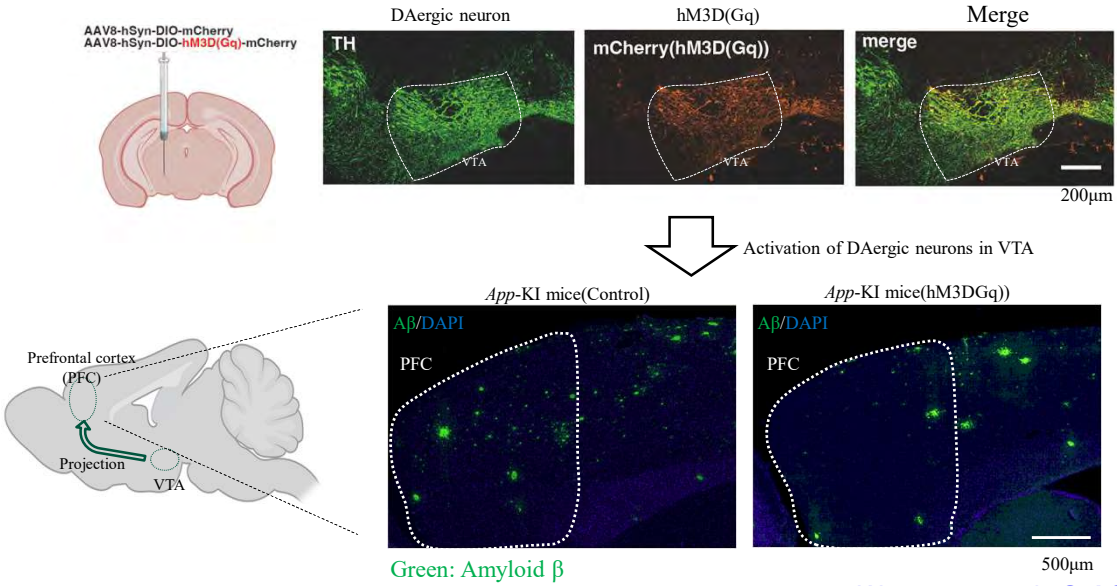
Deficiency of somatostatin receptor subtypes 1 and 4 down-regulates neprilysin expression and exacerbates Aβ pathology in the hippocampus of *App* knock-in mice.



Combination of SSTR subtypes 1 & 4 regulates neprilysin activity *in vivo*.

Nilsson *et al.*, under revision.

Another activator of neuronal neprilysin: dopaminergic neuron.

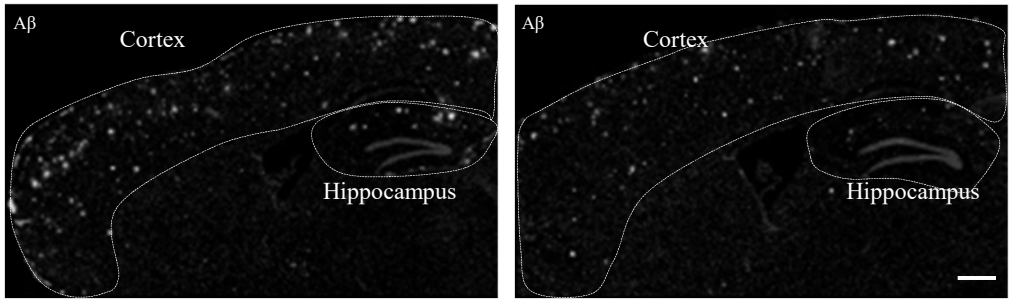


Green: Amyloid β
Blue: Nuclear

Watamura *et al.*, ***Sci Sig*** (2024)

Administration of L-Dopa attenuates Aβ pathology via upregulating neprilysin expression in the frontal cortex of *App* knock-in mice.

***App*-KI (Placebo)** ***App*-KI (L-dopa)**



This paper has received more than 100 media coverages in 19 countries.

Watamura *et al.*, ***Sci Sig*** (2024)

Differential roles of SST and DA neurons in regulating neprilysin in the brain

SSTR 1 & 4: Mainly in hippocampus (more important).
DA: Mainly in frontal cortex.

Combination of SST & DA receptor modulators will stop progression of preclinical AD.

Effect of neprilysin deficiency on neuropeptide levels in brain is relatively small.

Tissue	Neuropeptide	% Alteration (Average ± SE)
Cortex	Somatostatin	+19% ± 8.0
	Substance P	+3.3% ± 5.4
	Chlecystokinin	+16% ± 6.1
	Neuropeptide Y	-10% ± 4.1
Hippocampus	Somatostatin	+15% ± 4.6
	Substance P	+1.5% ± 4.8
	Chlecystokinin	+4.9% ± 5.5
	Neuropeptide Y	+1.2% ± 4.3

Neprilysin degrades Aβ at presynapse of the excitatory neurons, whereas most neuropeptides are secreted from inhibitory neurons.

Modeling FTDP-17 and Aβ-tau axis

Generation of human *MAPT* KI mice

Adult WT mouse

2N/4R 441aa
2N/3R 410aa
1N/4R 412aa
1N/3R 381aa
0N/4R 383aa
0N/3R 352aa

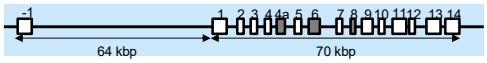
microtubule binding domain

MAPT KI

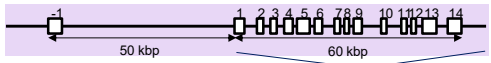
2N/4R 441aa
2N/3R 410aa
1N/4R 412aa
1N/3R 381aa
0N/4R 383aa
0N/3R 352aa

microtubule binding domain

Human *MAPT* Gene



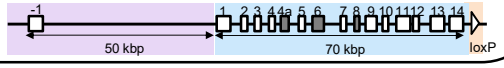
Mouse *Mapt* Gene



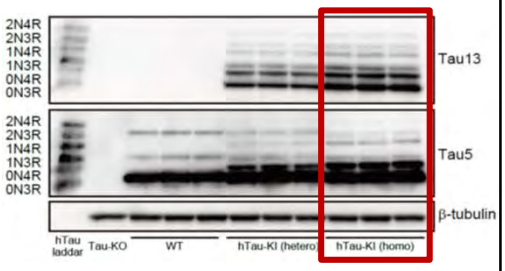
Targeting vector

x EII-Cre-Tg

***MAPT* KI**

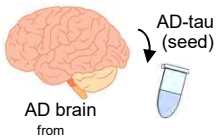


Expression of all six isoforms of tau



(Saito et al., 2019 JBC, Hashimoto et al., 2019 Nat Commun)

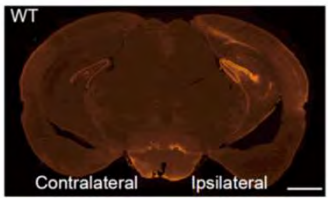
Generation of *MAPT* KI mice & propagation of pathological tau

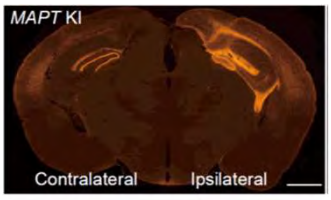


AD brain
from
Drs. V. Lee & J. Trojanwski

AD-tau
(seed)

Injection of 2μg AD-tau to
cortex and hippocampus



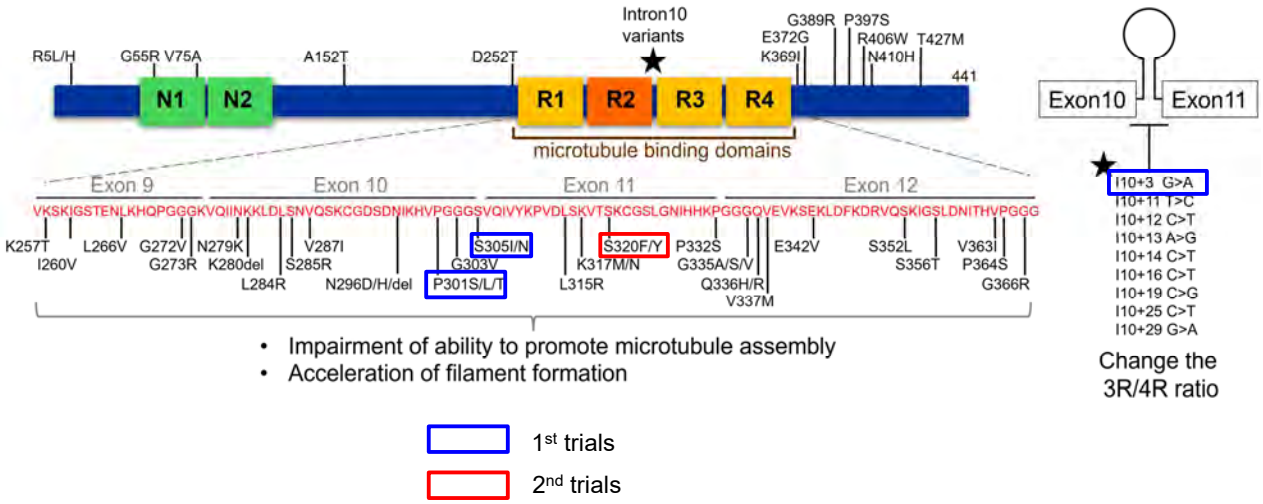


(Saito et al., 2019)

***MAPT* KI mice are more useful than WT mice for understanding the pathogenesis of tauopathy under human-like physiological conditions.**

RIKEN CBS

Pathogenic mutations in *MAPT* gene



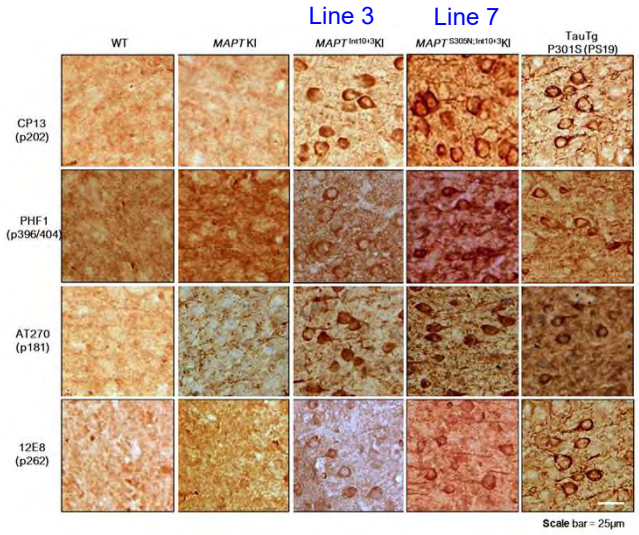
Watamura *et al.*, ***Nat Neurosci***, in press; Morito *et al.*, submitted.

Single, Double & triple mutant *MAPT* knock-in mouse lines

1. P301S
2. P301L
3. Int10+3 (plus *App*$^{NL-G-F}$)
4. P301S/Int10+3 (plus *App*$^{NL-G-F}$)
5. P301L/Int10+3
6. P301S/S320F
7. **S305N/Int10+3**
8. S305N/Int10+3/S320F
9. S305N/Int10+3/S320Y
10. P301S/Int10+3/S320F

Watamura *et al.*, ***Nat Neurosci***, in press; Morito *et al.*, under review.

Pathological accumulation of phosphorylated tau in *MAPT* knock-in mouse lines (15 moths old)



Saido, 2024; Watamura *et al.*, ***Nat Neurosci***, in press.

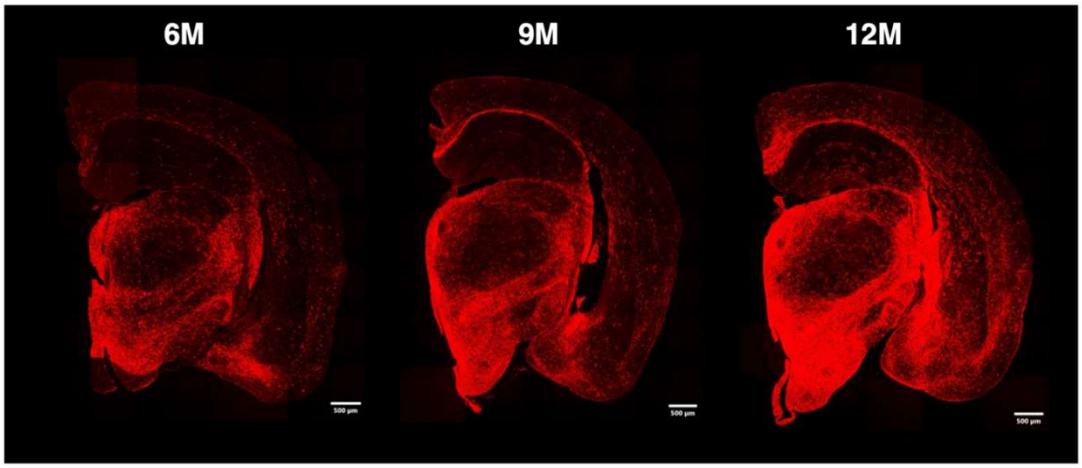
Single, Double & triple mutant *MAPT* knock-in mouse lines

1. P301S
2. P301L
3. Int10+3 (plus *App*$^{NL-G-F}$)
4. P301S/Int10+3 (plus *App*$^{NL-G-F}$)
5. P301L/Int10+3
6. P301S/S320F
7. S305N/Int10+3
8. S305N/Int10+3/S320F
9. S305N/Int10+3/S320Y
10. **P301S/Int10+3/S320F**

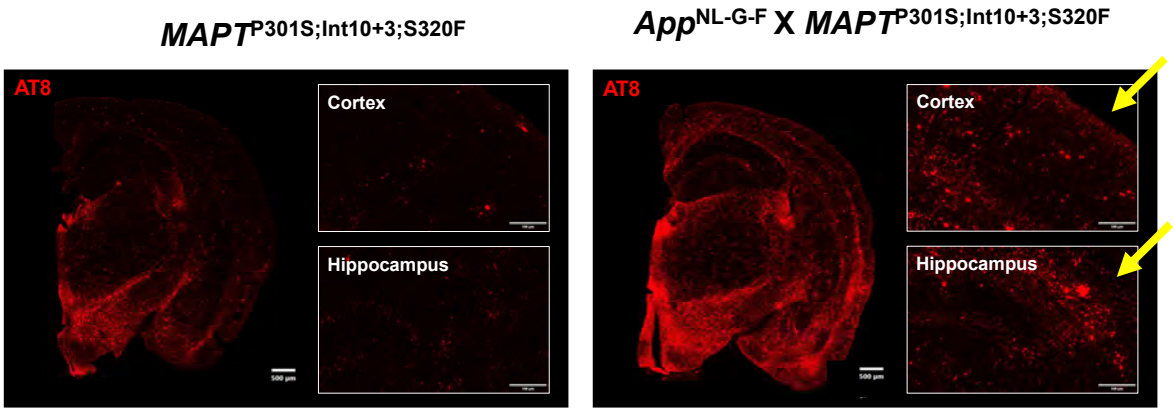
The combination of P301S/L and S320F mutations is known to synergistically promote tau fibrilization (Chen *et al.*, ***Nat Commun***, 2023).

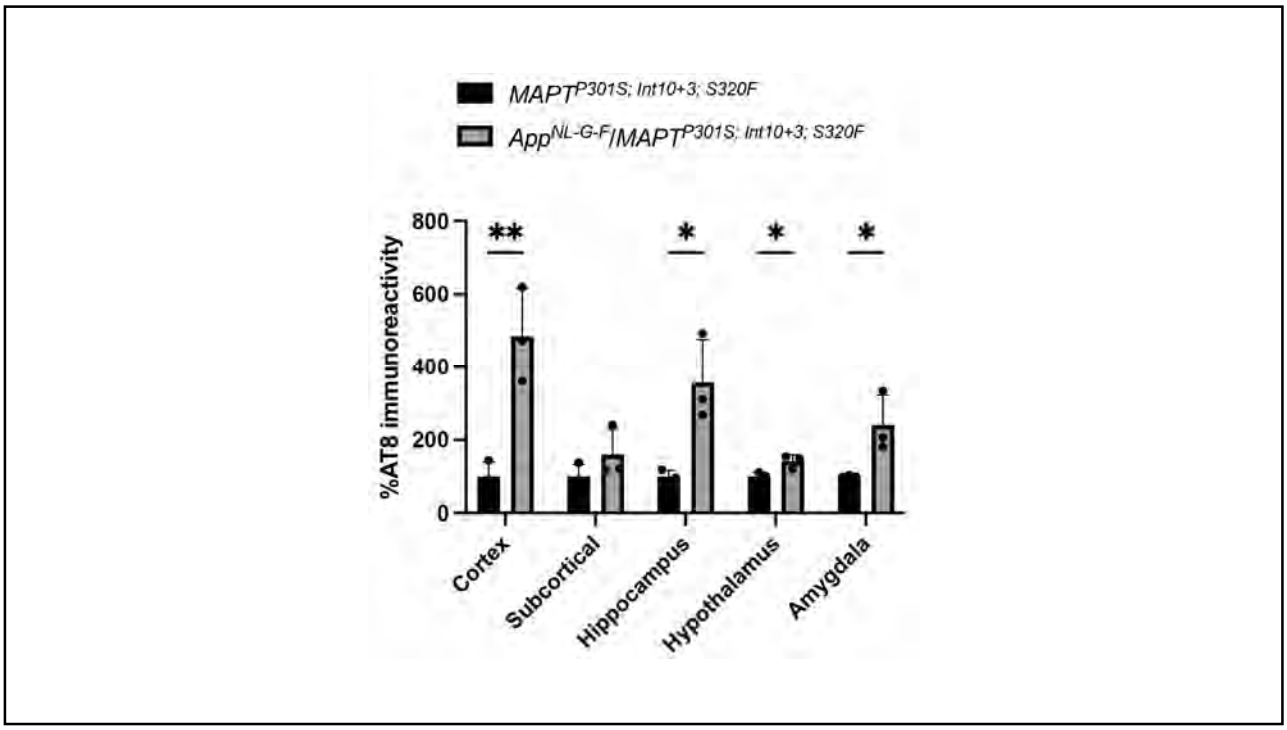
Watamura *et al.* (*Nat Neurosci*, in press); Morito *et al.*, under review.

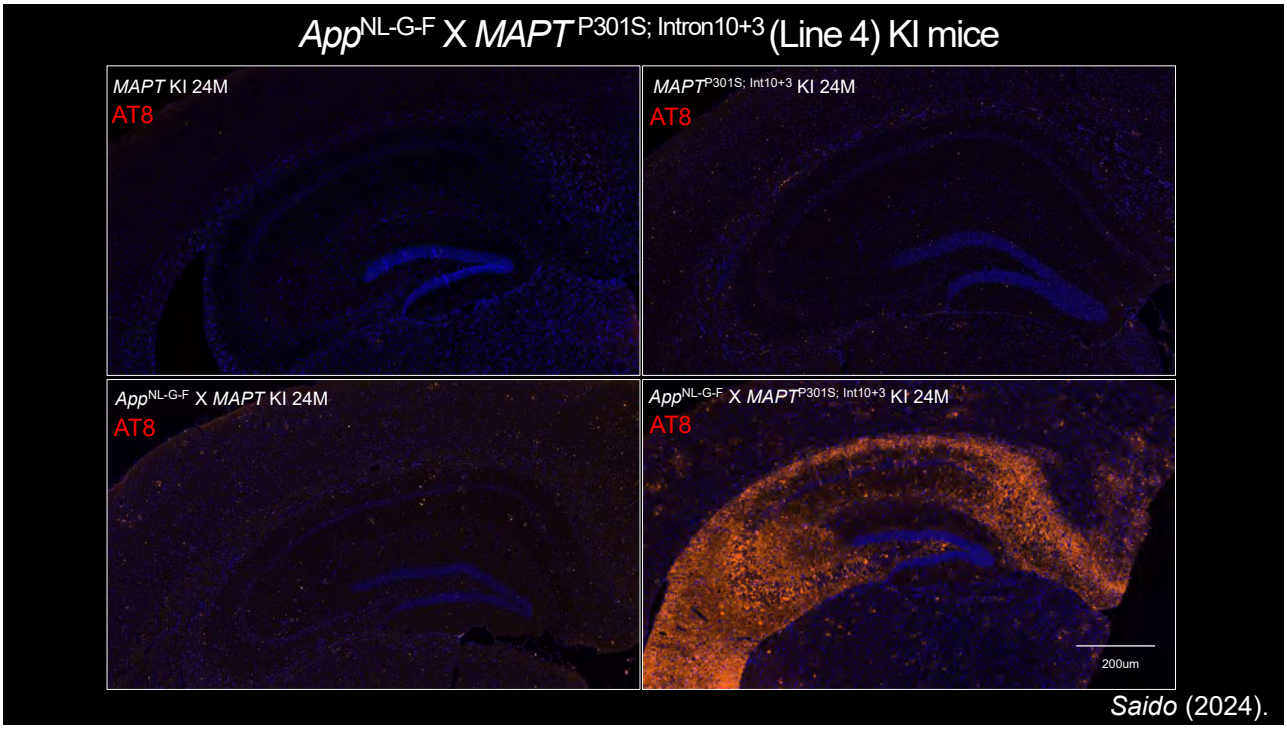
Tau pathology (AT8) in the next-generation model of FTD



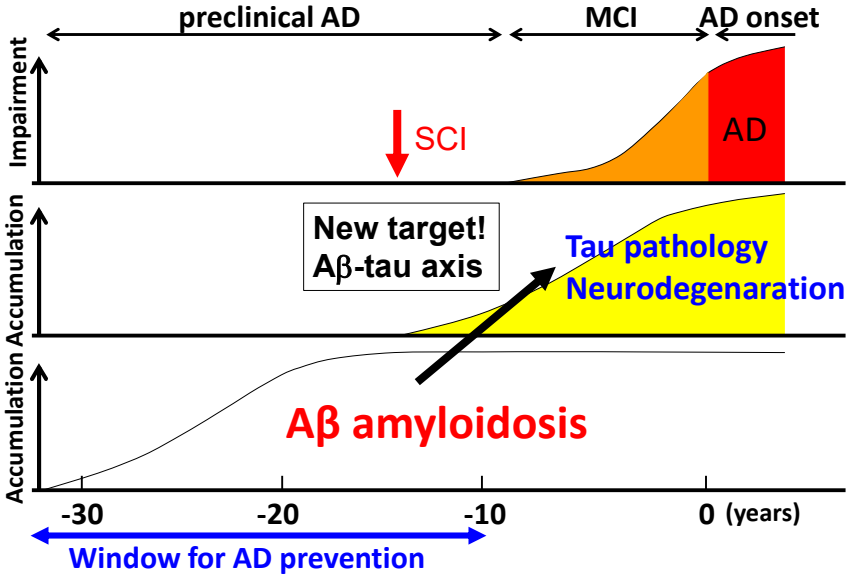
Watamura *et al.*, *Nat Neurosci,* in press; Morito *et al., Neuron*, under revision.




MAPT P301S; Int10+3; S320F
App NL-G-F/MAPT P301S; Int10+3; S320F
%AT8 immunoreactivity
800
600
400
200
0
**
*
*
*
Cortex
Subcortical
Hippocampus
Hypothalamus
Amygdala

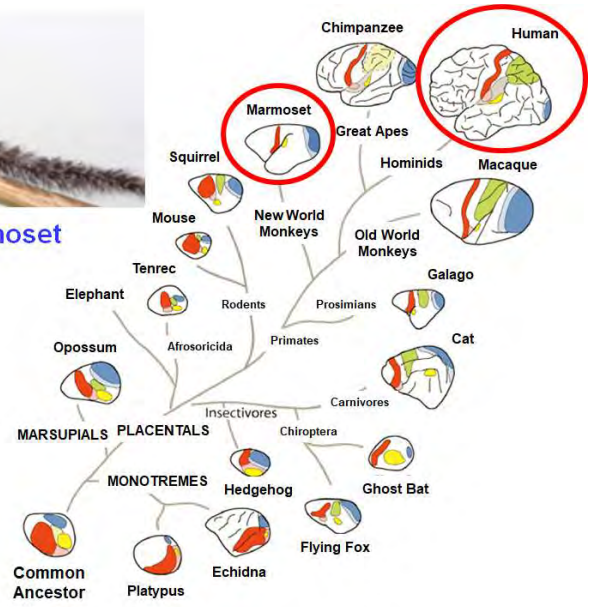

App NL-G-F X MAPT P301S; Intron10+3 (Line 4) KI mice
MAPT KI 24M
AT8
MAPT P301S; Int10+3 KI 24M
AT8
App NL-G-F X MAPT KI 24M
AT8
App NL-G-F X MAPT P301S; Int10+3 KI 24M
AT8
200um
Saido (2024).

Pathological and neurological chronology leading to AD



A brief additional topic for the future possibility: non-human primate model of AD

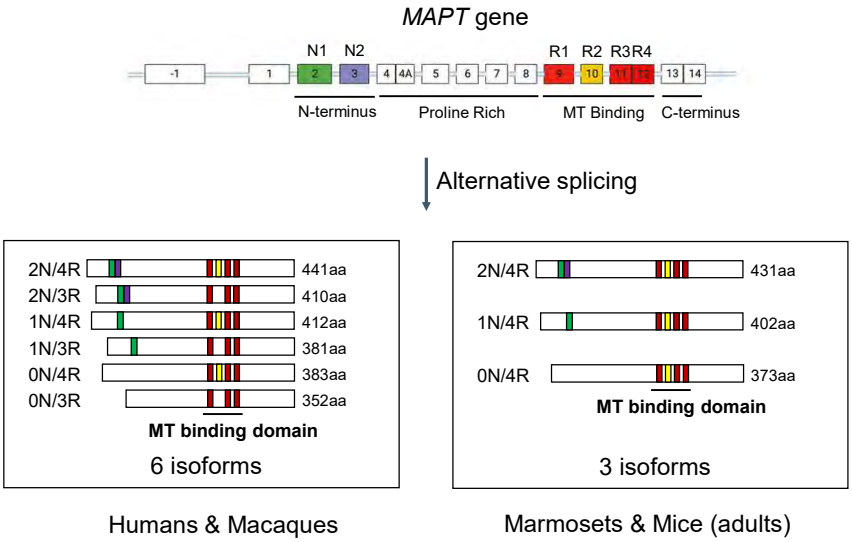
Common marmoset



Merits of using marmosets as a model for AD

1		Aβ: identical to human Aβ sequence.
2		**Aβ deposition with aging (7 years).**
3		**Phospho-tau accumulation with aging (12 years).**
4		Expression of 3 isoforms of tau (4 repeat)
5		*APOE* genotype: *APOE4*
6		High homology of GWAS-idntified risk genes
7		Immune systems similar to humans.
8		Diabetes with aging.
9		Human-like cognitive behaviors.
10		Visual and auditory communications.
11		Small: 350-500 g.
12		Relatively easy access to CSF.
13		Relatively short pregnancy: 145 days.
14		Non-REM and probable REM cycles during sleep.

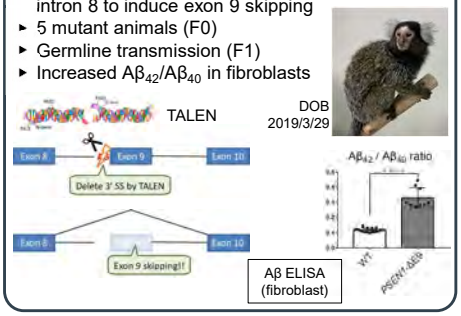
Adult marmoset's tau composed of 4R tau only is more pathogenic than macaque's tau of 3R/4R.



New AD models of non-human primates

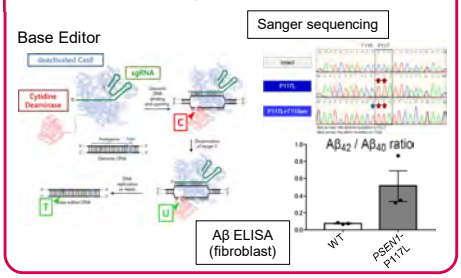
PSEN1-ΔE9 marmoset

- Familial AD (age of onset; 40~50 y/o)
- TALEN-mediated deletion of 3' splice site of intron 8 to induce exon 9 skipping
- 5 mutant animals (F0)
- Germline transmission (F1)
- Increased $A\beta_{42}/A\beta_{40}$ in fibroblasts



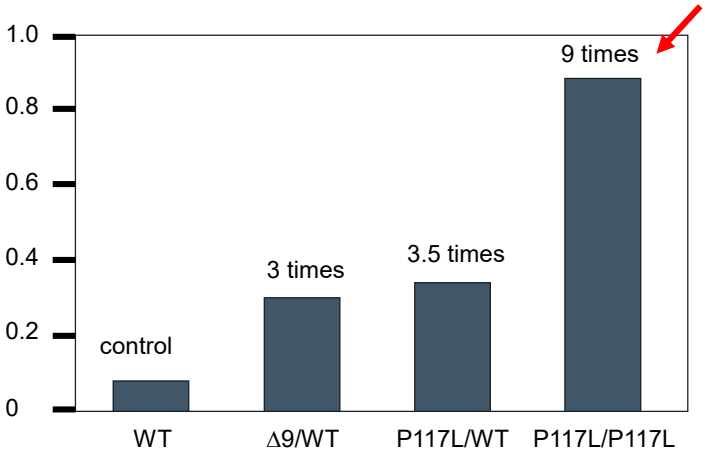
PSEN1-P117L marmoset

- Familial AD (age of onset; 30 y/o)
- Base Editor-mediated introduction of P117L
- 9 mutant animals (F0)
- Increased $A\beta_{42}/A\beta_{40}$ in fibroblasts



Sato *et al.* ***LabAnimal***, 2024; Kumita *et al*, in preparation.

Ratio of $A\beta_{42}/A\beta_{40}$ in model marmosets



The ratio inversely correlates with age-of-onset in humans.

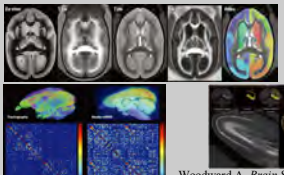
Sato *et al.* ***LabAnimal***, 2024; *Kumita et al*, in preparation.

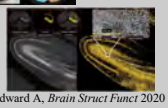
Future directions for AD marmoset research

Innovative technologies

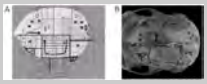
- High resolution MRI, functional MRI
- PET (amyloid, tau, TSPO)
- Biomarkers (Simoa)
- High throughput analysis (Proteomics, scRNA-seq, etc)
- Physiological analysis (EEG)
- Behavior analysis utilizing AI
- Tracer injections, etc.

Okano H, *Annu Rev Neurosci* 2021

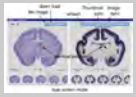




Woodward A, *Brain Struct Funct* 2020



Komatsu M, *J Vis Exp* 2019



Shimogori T, *Neurosci Res* 2018

Mutant *PSEN1* marmosets

Early amyloid pathology & behavioral abnormalities

Default mode network (DMN)

Sleep disturbance

Neuronal hyperexcitability

Making the mutants available to research community → We need resources to expand colonies!

Application to "near-clinical" studies in the future → Substantial reduction of the risk of clinical trials

Sato *et al.* ***LabAnimal***, 2024; *Kumita et al*, in preparation.

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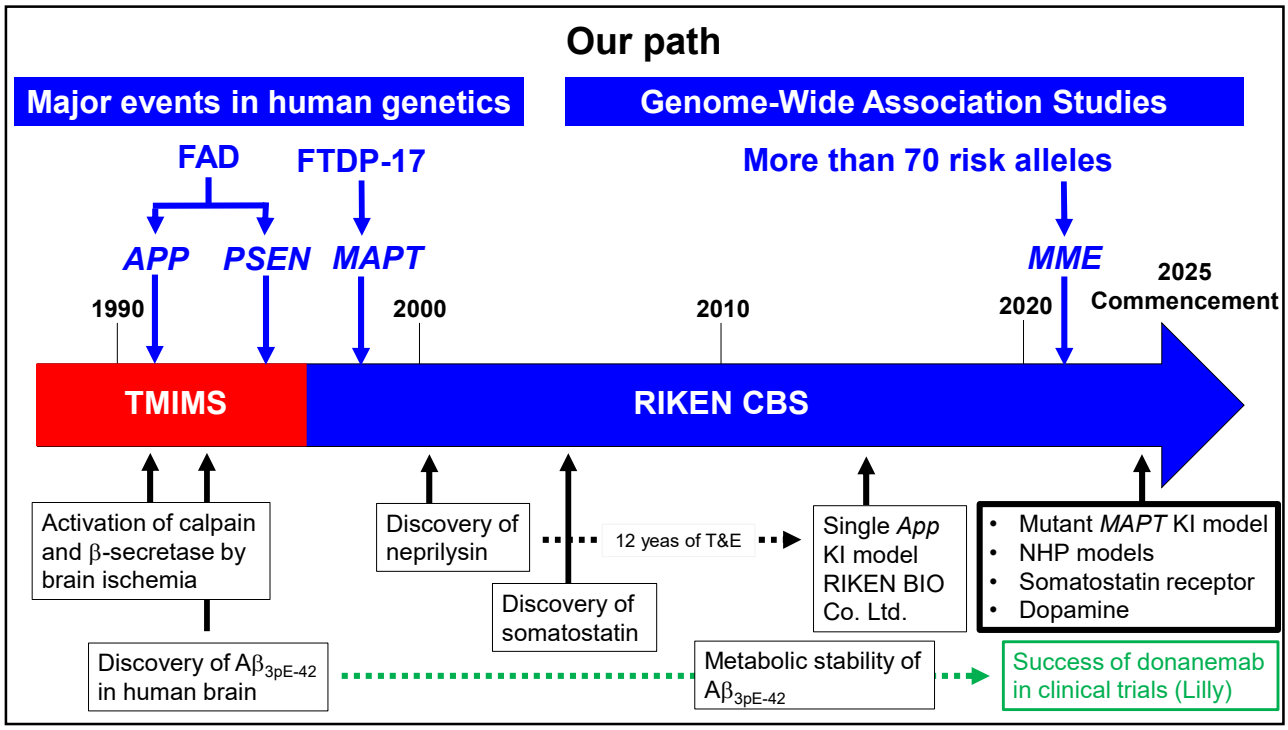

If I need to do these outside RIKEN, the R&D will be delayed for more than 2 years because the transfer and breeding of model animals will take a lot of time. Besides, animal facilities in most other organizations are insufficient.

However, there is a solution for this…..

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Participating company(es) will share the intellectual properties to be generated with RIKEN.

RIKEN CBS-Company Collaboration Center for preclinical AD research

Four Laboratories ← **RIKEN BIO (RIKEN Innovation)**

1. GPCR medications

1. *In silico* screening
2. *In vitro* screening
3. Negative screening
4. *In vivo* screening
5. Pharmacodynamic optimization
6. Validation using NHP models

2. Tau pathology

1. PROTAC
2. Anti-oligomerization
3. *In vitro* screening
4. *In vivo* screening
5. Pharmacodynamic optimization
6. Validation using NHP models

3. Omics

1. Aβ-tau axis models
2. Microscopic omics
3. AAV-ShRNA validation
4. Identification of new targets
5. Plasma and genetic biomarkers
6. Validation using human samples

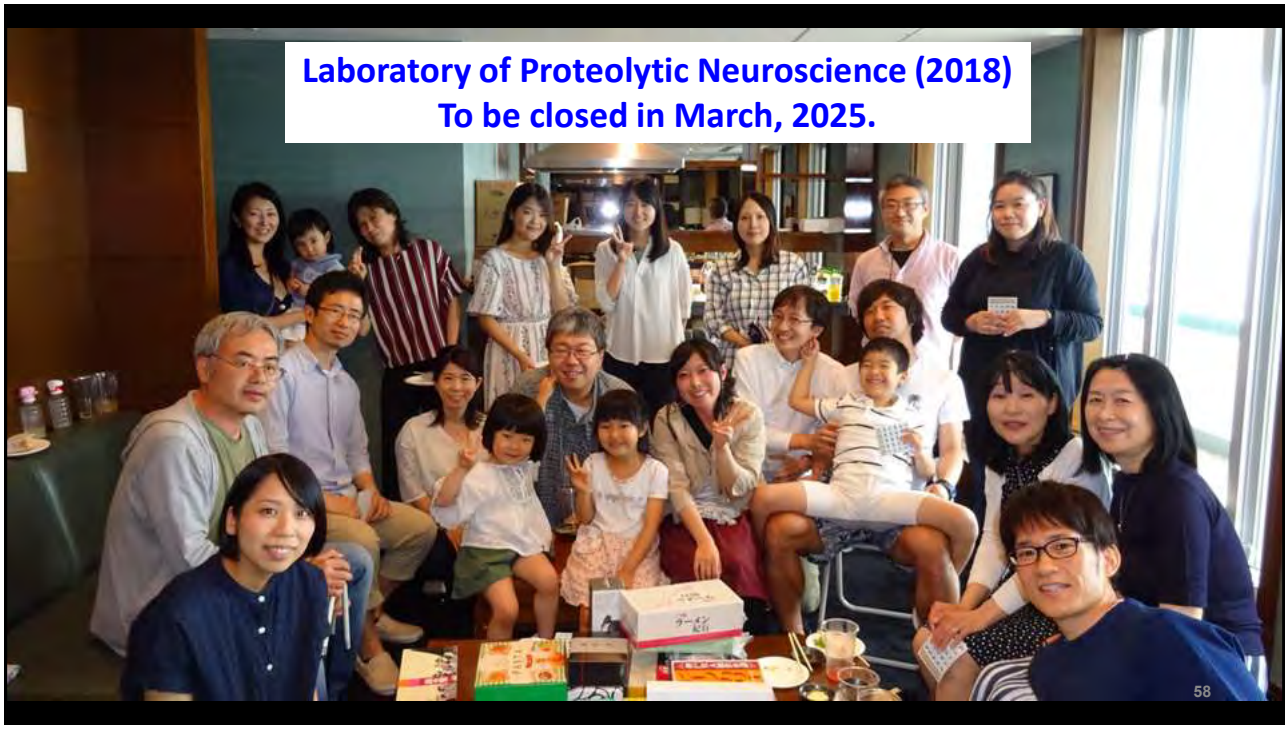
4. Nonhuman primate models

1. Expansion of colonies (*PSEN1*$^{P117L/P117L}$)
2. Biomarkers (Diagnosis & prognosis
3. Near-clinical tests
4. Generation of FTD (P301S or S320F) models

Participating company(es) will share the intellectual properties to be generated with RIKEN.

Business Model of RIKEN BIO Co. Ltd.

1. Licensing of mouse models of dementia.
2. Registration of a trade mark "NEGATIVE DATA™" for the days of AI-assisted publication (Japan, USA & China)
3. Resources for the "RIKEN CBS-Company Collaboration Center for preclinical AD research" by stock transfer→Generation of more than 10-fold profits in several years.
4. International patents for somatostatin receptor
5. Plasma biomarkers for preclinical AD, tau pathology and the Aβ-tau axis.
6. GPCR medications by *in silico, in vitro* and *in vivo* screening: Collaboration with megapharmas



Laboratory for Proteolytic Neuroscience, March 10, 2023.
We were the most cited in RIKEN CBS in 2022 & 2023.
THE PNS LABORATORY WILL CLOSE ON MARCH 31, 2025.

And, for the futures of colleagues and their families!

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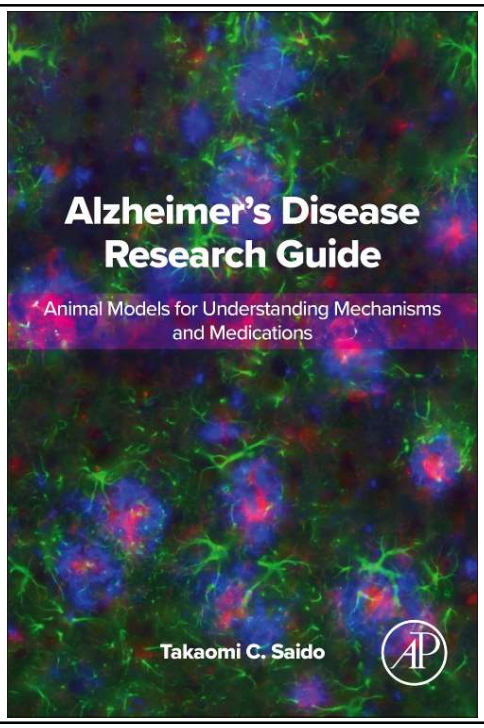
Major publications from PNS Lab

1. Saido TC *et al.* (1995) Dominant and differential deposition of distinct β-amyloid peptide species, $A\beta_{N3(pE)}$, in senile plaques. ***Neuron***, 14, 457-466.
2. Iwata et *al.* (2000) Identification of the major $A\beta_{1-42}$-degrading catabolic pathway in brain parenchyma: Suppression leads to biochemical and pathological deposition. ***Nat Med***, 6, 143-151.
3. Iwata *et al.* (2001). Metabolic regulation of brain Aβ by neprilysin. ***Science***, 292, 1550-1552.
4. Tsubuki S, Takaki Y, Saido TC. (2003) Dutch, Flemish, Italian, and Arctic mutations of APP and resistance of Aβ to physiologically relevant proteolytic degradation. ***Lancet***, 361, 1957-1958.
5. Saito *et al.* (2005) Somatostatin regulates brain amyloid β peptide, $A\beta_{42}$, through modulation of proteolytic degradation. ***Nat Med***, 11, 434-439.
6. Saito *et al.* (2014) Single *App* knock-in mouse models of Alzheimer's disease. ***Nat Neurosci***, 17, 661-663.
7. Watamura *et al.* (2022) Somatostatin-evoked Aβ catabolism in the brain: Mechanistic involvement of α-endosulfine-K_{ATP} channel pathway. ***Mol Psychiatry***, 27, 1816–1828.
8. Saido (2024) ***Alzheimer's Disease Research Guide: Animal Models for Understanding Mechanisms and Medications,*** Elsevier.
9. Watamura *et al.* (2024) The dopaminergic system regulates amyloid-ß dynamics in the brain through neprilysin-mediated proteolytic degradation. ***Science Signaling***, doi: 10.1126/scisignal.adk1822.
10. Iwata *et al.* (2024) Metabolic resistance of Aβ3pE-42, target epitope of the anti-Alzheimer therapeutic antibody, donanemab. ***Life Sci Alliance,*** in press.
11. Sato *et al.* (2024) Production of a heterozygous exon skipping model of common marmosets using gene-editing technology. ***LabAnimal,*** in press.
12. Watamura *et al.* (2024) *In vivo* hyperphosphorylation of tau is associated with synaptic loss and behavioral abnormalities in the absence of tau seeds, ***Nat Neurosci***, in press.



KEY FEATURES

- Reviews why familial Alzheimer's disease is vital to understanding sporadic Alzheimer's disease
- Describes the latest "game changer" animal models of Alzheimer's disease and frontotemporal dementia in detail
- Explains how various Alzheimer's disease medications have failed clinical trials
- Discusses pros and cons of therapeutic antibodies, lecanemab and donanemab, that were recently found to be effective in recent clinical trials
- Details the application of genome editing as a treatment for familial Alzheimer's disease
- Proposes publishing "*Journal of Negative Data*" for the days of generative AI-assisted publication, AI being unable to distinguish between reproducible and unreproducible data, particularly important in Alzheimer's research

DESCRIPTION

Alzheimer's Disease Research Guide: Animal Models for Understanding Mechanisms and Medications provides researchers with a comprehensive guide, detailing every aspect of Alzheimer's Disease research, including chapters on neuroinflammation, immunotherapy, biomarkers, and animal modeling. This book begins with historical perspectives of both pathological chronology and pathological biochemistry in relation to Alzheimer's disease. Other chapters review Amyloidogenic AB and Non-Amyloidogenic tau and Metabolism of AB major components to the research and understanding of Alzheimer's research. The book concludes with specific treatment chapters including how to develop safe, effective, and inexpensive medications and the application of genome editing to the treatment of Familial Alzheimer's Disease.

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