



Recent Advances in the Modeling of Alzheimer's Disease

Hiroki Sasaguri¹, Shoko Hashimoto¹, Naoto Watamura¹, Kaori Sato^{1,2}, Risa Takamura^{1,2}, Kenichi Nagata³, Satoshi Tsubuki¹, Toshio Ohshima², Atsushi Yoshiki⁴, Kenya Sato^{1,5}, Wakako Kumita^{1,5}, Erika Sasaki^{5,6}, Shinobu Kitazume⁷, Per Nilsson⁸, Bengt Winblad⁸, Takashi Saito^{9,10*}, Nobuhisa Iwata^{11*} and Takaomi C. Saido^{1*}

¹ Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science, Wako, Japan, ² Laboratory for Molecular Brain Science, Department of Life Science and Medical Bioscience, Waseda University, Shinjuku City, Japan, ³ Department of Functional Anatomy and Neuroscience, Nagoya University Graduate School of Medicine, Nagoya, Japan, ⁴ Experimental Animal Division, RIKEN BioResource Research Center, Tsukuba, Japan, ⁵ Department of Marmoset Biology and Medicine, Central Institute for Experimental Animals, Kawasaki, Japan, ⁶ Laboratory for Marmoset Neural Architecture, RIKEN Center for Brain Science, Wako, Japan, ⁷ Department of Clinical Laboratory Sciences, School of Health Sciences, Fukushima Medical University, Fukushima, Japan, ⁸ Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Stockholm, Sweden, ⁹ Department of Neurocognitive Science, Institute of Brain Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, ¹⁰ Department of Neuroscience and Pathobiology, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan, ¹¹ Department of Genome-Based Drug Discovery and Leading Medical Research Core Unit, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

OPEN ACCESS

Edited by:

Brian Hansen,
Aarhus University, Denmark

Reviewed by:

Eugenio Gutiérrez Jiménez,
Aarhus University, Denmark
Yingjun Zhao,
Xiamen University, China

*Correspondence:

Takashi Saito
saito-t@med.nagoya-cu.ac.jp
Nobuhisa Iwata
iwata-n@nagasaki-u.ac.jp
Takaomi C. Saido
takaomi.saido@riken.jp

Specialty section:

This article was submitted to
Neurodegeneration,
a section of the journal
Frontiers in Neuroscience

Received: 02 November 2021

Accepted: 22 February 2022

Published: 31 March 2022

Citation:

Sasaguri H, Hashimoto S, Watamura N, Sato K, Takamura R, Nagata K, Tsubuki S, Ohshima T, Yoshiki A, Sato K, Kumita W, Sasaki E, Kitazume S, Nilsson P, Winblad B, Saito T, Iwata N and Saido TC (2022) Recent Advances in the Modeling of Alzheimer's Disease. *Front. Neurosci.* 16:807473. doi: 10.3389/fnins.2022.807473

Since 1995, more than 100 transgenic (Tg) mouse models of Alzheimer's disease (AD) have been generated in which mutant amyloid precursor protein (APP) or APP/presenilin 1 (PS1) cDNA is overexpressed (**1st generation models**). Although many of these models successfully recapitulate major pathological hallmarks of the disease such as amyloid β peptide (A β) deposition and neuroinflammation, they have suffered from artificial phenotypes in the form of overproduced or mislocalized APP/PS1 and their functional fragments, as well as calpastatin deficiency-induced early lethality, calpain activation, neuronal cell death without tau pathology, endoplasmic reticulum stresses, and inflammasome involvement. Such artifacts bring two important uncertainties into play, these being (1) why the artifacts arise, and (2) how they affect the interpretation of experimental results. In addition, destruction of endogenous gene loci in some Tg lines by transgenes has been reported. To overcome these concerns, single *App* knock-in mouse models harboring the Swedish and Beyreuther/Iberian mutations with or without the Arctic mutation (*App*^{NL-G-F} and *App*^{NL-F} mice) were developed (**2nd generation models**). While these models are interesting given that they exhibit A β pathology, neuroinflammation, and cognitive impairment in an age-dependent manner, the model with the Arctic mutation, which exhibits an extensive pathology as early as 6 months of age, is not suitable for investigating A β metabolism and clearance because the A β in this model is resistant to proteolytic degradation and is therefore prone to aggregation. Moreover, it cannot be used for preclinical immunotherapy studies owing to the discrete affinity it shows for anti-A β antibodies. The weakness of the latter model (without the Arctic mutation) is that the pathology may require up to 18 months before it becomes sufficiently apparent for experimental investigation.

Nevertheless, this model was successfully applied to modulating A β pathology by genome editing, to revealing the differential roles of neprilysin and insulin-degrading enzyme in A β metabolism, and to identifying somatostatin receptor subtypes involved in A β degradation by neprilysin. In addition to discussing these issues, we also provide here a technical guide for the application of *App* knock-in mice to AD research. Subsequently, a new double knock-in line carrying the *App*^{NL-F} and *Psen1*^{P117L/WT} mutations was generated, the pathogenic effect of which was found to be synergistic. A characteristic of this **3rd generation model** is that it exhibits more cored plaque pathology and neuroinflammation than the *App*^{NL-G-F} line, and thus is more suitable for preclinical studies of disease-modifying medications targeting A β . Furthermore, a derivative *App*^{G-F} line devoid of Swedish mutations which can be utilized for preclinical studies of β -secretase modifier(s) was recently created. In addition, we introduce a new model of cerebral amyloid angiopathy that may be useful for analyzing amyloid-related imaging abnormalities that can be caused by anti-A β immunotherapy. Use of the *App* knock-in mice also led to identification of the α -endosulfine-K_{ATP} channel pathway as components of the somatostatin-evoked physiological mechanisms that reduce A β deposition *via* the activation of neprilysin. Such advances have provided new insights for the prevention and treatment of preclinical AD. Because tau pathology plays an essential role in AD pathogenesis, knock-in mice with human tau wherein the entire murine *Mapt* gene has been humanized were generated. Using these mice, the carboxy-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON) was discovered as a mediator linking tau pathology to neurodegeneration and showed that tau humanization promoted pathological tau propagation. Finally, we describe and discuss the current status of mutant human tau knock-in mice and a non-human primate model of AD that we have successfully created.

Keywords: Alzheimer's disease, amyloid – beta, amyloidosis, tau propagation, somatostatin, mouse model, non-human primate (NHP)

1st, 2nd, AND 3rd GENERATION MOUSE MODELS OF ALZHEIMER'S DISEASE

The deposition of amyloid β peptide (A β) in the brain is the major pathological hallmark of Alzheimer's disease (AD), which is considered the most common type of dementia in the world (Karran and De Strooper, 2016; Selkoe and Hardy, 2016). To date, disease-associated mutations in the *presenilin 1* (*PSEN1*) and *presenilin 2* (*PSEN2*) genes number more than 300, while more than 50 mutations have been reported in the *amyloid precursor protein* (*APP*) gene (Alzforum¹). In response to these findings, many transgenic mouse models overexpressing mutant APP or APP/PSEN1 cDNAs have been developed (**1st generation models**) (Sasaguri et al., 2017), however they often suffer from experimental limitations resulting from the mislocalization of APP (**Figure 1**) and by the overproduction of APP fragments such as the C-terminal fragment of APP generated by β -secretase (CTF- β) and APP intracellular domain (AICD). Neither of these fragments appears to accumulate in AD brains, meaning that artificial endosomal abnormalities

(Kwart et al., 2019) and transcriptional malfunctions (Nalivaeva et al., 2014), respectively, may be induced. Other overexpression artifacts include calpain activation (Saito et al., 2016), calpastatin deficiency-induced early lethality (Higuchi et al., 2012), and endoplasmic reticulum stresses (Hashimoto et al., 2018). Furthermore, it was demonstrated that the random insertion of transgene(s) resulted in the destruction of unexpectedly large regions of endogenous gene loci in the host animal (Gamache et al., 2019). We suggest that all transgenic mouse models being used in research in which APP or APP/PSEN1 are overexpressed should undergo whole genome sequencing (WGS) so that destroyed loci that possibly affect their phenotypes can be identified (Sasaguri et al., 2017).

To overcome these drawbacks, single *App* knock-in mice, i.e., *App*^{NL-G-F/NLG-F} knock-in (*App*^{NL-G-F}) and *App*^{NL-F/NL-F} knock-in (*App*^{NL-F}) lines, were generated that harbor the Swedish (KM670/671NL) (Citron et al., 1992; Mullan et al., 1992) and Beyreuther/Iberian (I716F) (Lichtenthaler et al., 1999) mutations with or without the Arctic (E693G) (Nilsberth et al., 2001) mutation (**2nd generation models**) (**Figure 2**) (Saito et al., 2014; Sasaguri et al., 2017). These mice, which exhibit typical A β pathology, neuroinflammation and memory impairment

¹<http://www.alzforum.org>

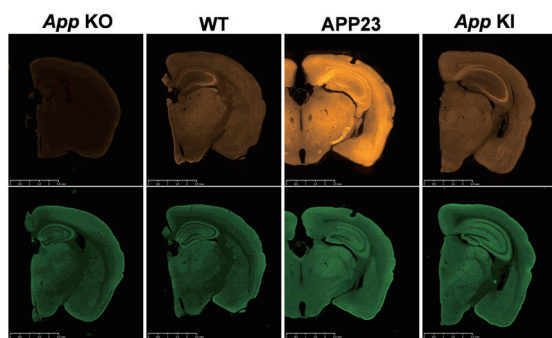


FIGURE 1 | Mislocalization of APP in APP-overexpressing mice. *App* KO mice, WT mice, APP23 (APP-overexpressing mice) and *App* KI mice (*App*^{NL-F/NL-F}) were subjected to immunohistochemistry using antibodies to APP, 22C11 (**upper panels**) and synaptophysin, a synaptic vesicle marker (**lower panels**) as indicated. *App* KO mice were used as negative controls for APP staining. While APP is selectively expressed in the axons of WT and KI mice, APP23 expresses unphysiologically high levels of APP not only in the axons but also in the soma and dendrites. The scale bar indicates 2 mm.

(Saito et al., 2014; Masuda et al., 2016), are being used in more than 500 research laboratories world-wide. At present, the *App*^{NL-G-F} line is being used more frequently than the *App*^{NL-F} line given that it develops A β pathology approximately three times faster (Saito et al., 2014) and can be used to analyze downstream events such as neuroinflammation (Shirotani et al., 2019; Chen et al., 2020; Sobue et al., 2021), pericyte signaling (Nortley et al., 2019), oxidative stress (Hashimoto et al., 2019; Hongo et al., 2020; Urano et al., 2020), tau propagation (Saito et al., 2019), and spatial memory impairment (Masuda et al., 2016; Jun et al., 2020; Sutoko et al., 2021; **Table 1**). Human Arctic mutation carriers are indistinguishable from other familial and sporadic AD patients in pathological and neurological terms except for low retention of ¹¹C-labeled Pittsburgh compound B (PiB) in PET study (Basun et al., 2008), indicating that the mutant mice are relevant models for studying AD in general.

In addition to *App*^{NL-F} and *App*^{NL-G-F} models, *App* knock-in mice devoid of the Swedish mutations (*App*^{G-F} mice) have been recently developed, in which the Swedish mutations (NL) were replaced by a wild-type sequence (KM) (**Figure 3** and **Table 2**). The *App*^{G-F} mice are more suitable for preclinical studies of β -secretase inhibition given that the Swedish mutation affects the reactivity of APP to β -site amyloid precursor protein cleaving enzyme 1 (BACE1) and most AD patients do not carry Swedish mutations (Watanabe et al., 2021b).

Despite the advantages mentioned above, the *App*^{NL-G-F} line is not suitable for investigating the metabolism, clearance or deposition of A β because the Arctic mutation present in the middle of the A β sequence results in an A β that is resistant to proteolytic degradation (Tsubuki et al., 2003) and susceptible to aggregation (Nilsberth et al., 2001). Moreover, this model is not suitable for use in preclinical immunotherapy studies due to its affinity for anti-A β antibodies, even in the presence of guanidine hydrochloride (GuHCl) (Saito et al., 2014). The Arctic mutation may also directly or indirectly interfere with interactions between A β deposition and the apolipoprotein E

genotype (Morishima-Kawashima et al., 2000), although there is no experimental evidence for this. In contrast, the *App*^{NL-F} line accumulates wild-type human A β , but it may take up to 18 months for the pathology to become sufficiently evident for investigational studies to be carried out (Saito et al., 2014), which is too long for researchers to wait in a practical sense. Therefore, a new mouse model that accumulates wild-type human A β as quickly as the *App*^{NL-G-F} model, but did not depend on the presence of the Arctic mutation was desired.

To achieve this, the heterozygous *Psen1*^{P117L/WT} mutant line (*Psen1*^{P117L}) which, of the several *Psen1* mutants, exhibits the largest increase in A β ₄₂/A β ₄₀ ratio in the brain (Sasaguri et al., 2018) was utilized. The *Psen1*^{P117L} line was generated by base editing technology (Komor et al., 2016). The *App*^{NL-F} mice were crossed with *Psen1*^{P117L} mice, despite it being unclear whether their pathogenic effects, both of which act on the γ -cleavage of CTF- β , would be additive or not *in vivo* (**Figure 4**). The pathological phenotypes of *App*^{NL-F} mice were markedly enhanced in a synergistic manner with the *Psen1*^{P117L} mutation (Sato et al., 2021), with *App*^{NL-F} X *Psen1*^{P117L/WT} mice showing a more aggressive cored plaque pathology and neuroinflammation than the *App*^{NL-G-F} mice (**Figure 5**). These double mutant mice (**3rd generation model**) will likely become highly relevant tools for examining the pathologic mechanisms upstream of A β deposition. Moreover, these mice can be highly useful for the preclinical screening of disease-modifying therapy candidates promoting A β degradation or disaggregation, without the added concern associated with artificial effects caused by the Arctic mutation. We expect the double homozygous line, *App*^{NL-F} X *Psen1*^{P117L/P117L}, to exhibit an even more-aggressive pathology. In any case, the *App*^{NL-F} X *Psen1*^{P117L} mice are superior to the *App*^{NL-G-F} mice or the *App*^{NL-G-I} mice (Xia et al., 2021) for universal and unbiased drug screening particularly because the A β -degrading enzyme, neprilysin (NEP: Neutral endopeptidase), has become a therapeutic target. The *App*^{NL-G-I} mice are similarly designed as the *App*^{NL-G-F} mice, in which the Beyreuther/Iberian mutation was replaced by the Austrian mutation (Kumar-Singh et al., 2000). The characteristics of the *App* knock-in mouse lines are listed in **Table 3**.

PRECAUTIONS REGARDING THE UTILITY OF *App* KNOCK-IN MICE

There are several precautions to be aware of to make the best use of the *App* knock-in mice.

Nomenclature

A number of the *App* knock-in mouse users use incorrect nomenclature such as APP-NLF, APP^{NLF} and APP^{NL-F} instead of the *App*^{NL-F} mice, which accords with international rules of standard genomic nomenclature. Genetic names always need to be italicized.

Line-Ups and Biochemical Analyses

Approximately 20 lines of mutant mice, published or unpublished, can currently or in the very near future be

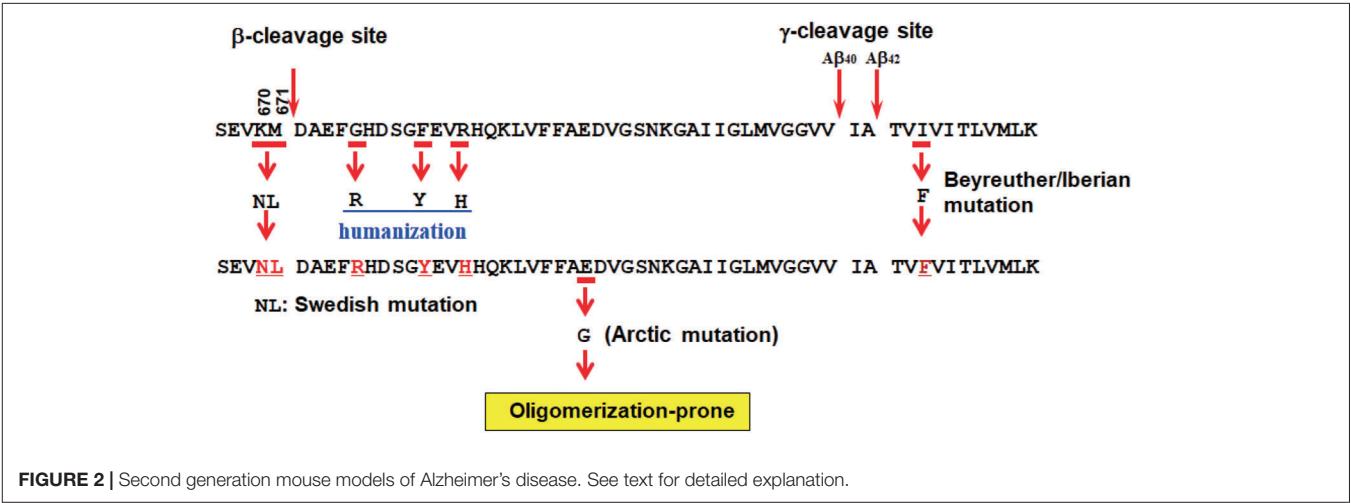
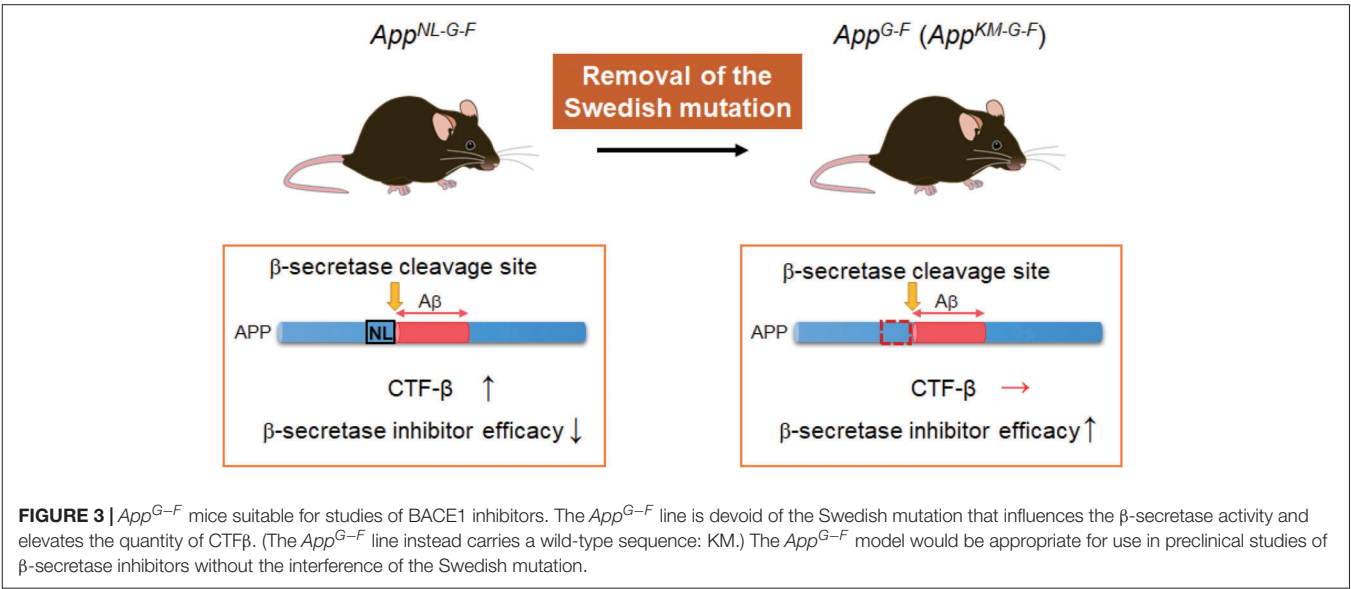


TABLE 1 | Successful application of the 2nd generation mouse models.

- (1) Behavioral analysis using IntelliCage (Masuda et al., 2016; Sutoko et al., 2021).
- (2) Three-dimensional visualization of amyloid pathology by transparency (Hama et al., 2015; Susaki et al., 2020).
- (3) Impairment of gamma oscillations in medial entorhinal cortex (Nakazono et al., 2017).
- (4) Additional genetic manipulation of the 2nd generation models through genome editing (Nagata et al., 2018; Watamura et al., 2021b).
- (5) Generation of the double knock-in mouse models (Hashimoto et al., 2019; Saito et al., 2019; Sato et al., 2021).
- (6) Assessment of vascular dysfunction in the 2nd generation models (Nortley et al., 2019; Tachida et al., 2020).
- (7) Assessment of sleep dysfunction in the 2nd generation models (Maezono et al., 2020).
- (8) Assessment of place cell dysfunction in the 2nd generation models (Jun et al., 2020; Takamura et al., 2021).
- (9) Analyses of various aspects of neuroinflammation (Shirotani et al., 2019; Chiasseu et al., 2020; Salobrar-García et al., 2020; Barrett et al., 2021; Sobue et al., 2021).
- (10) Application of spatial transcriptomics (Chen et al., 2020).



provided to academic and not-for-profit researchers for non-commercial research in a timely fashion with minimum restrictions (Table 2; Saito et al., 2014; Sasaguri et al., 2018). It is also recommended that scientists use optimized protocols for isolating Aβ from animal brain as previously described (Iwata et al., 2005; Figure 6). The method allows the most sensitive quantification of both soluble and insoluble Aβ with the smallest protocol deviations.

TABLE 2 | List of mutant mice that are and will be made available to the research community.

Strains	Gene(s)	Modification Information	Availability* ⁵	References* ⁵
<i>App</i> ^{NL} KI	<i>App</i> * ¹	KM670/671NL (Swedish)	RBRC* ⁶ (RBRC06342)	Saito et al., 2014
<i>App</i> ^{NL-F} KI	<i>App</i> * ¹	KM670/671NL (Swedish), I716F (Iberian/Beyreuther)	RBRC (RBRC06343)	Saito et al., 2014
<i>App</i> ^{NL-G-F} KI	<i>App</i> * ¹	KM670/671NL (Swedish), E693G (Arctic), I716F (Iberian/Beyreuther)	RBRC (RBRC06344)	Saito et al., 2014
<i>App</i> ^{G-F} KI	<i>App</i> * ¹	E693G (Arctic), I716F (Iberian/Beyreuther)	Soon to be available from RBRC	Watanura et al., 2021b
<i>App</i> ^{huAβ} KI	<i>App</i> * ¹	No mutation (humanized Aβ sequence)	Soon to be available from RBRC	Watanura et al., 2021b
<i>Psen1</i> ^{P436S} KI	<i>Psen1</i> * ²	P436S	Available* ⁷	Sasaguri et al., 2018
<i>Psen1</i> ^{P117L/A} KI	<i>Psen1</i> * ³	P117L/A	Available* ⁷	Sasaguri et al., 2018; Sato et al., 2021
<i>App</i> ^{NL-F} KI/ <i>Psen1</i> ^{P117L} KI	<i>App</i> * ¹ , <i>Psen1</i> * ³	<i>App</i> : KM670/671NL, I716F <i>Psen1</i> : P117L	Soon to be available from RBRC	Sato et al., 2021
<i>MAPT</i> KI	<i>MAPT</i> * ⁴	Humanization of the <i>Mapt</i> gene	RBRC (RBRC09995)	Hashimoto et al., 2019; Saito et al., 2019
<i>App</i> ^{NL} KI/ <i>hMAPT</i> KI	<i>App</i> * ¹ , <i>MAPT</i> * ⁴	<i>App</i> : KM670/671NL <i>MAPT</i> : No mutation	RBRC (RBRC10041)	Saito et al., 2019
<i>App</i> ^{NL-F} KI/ <i>hMAPT</i> KI	<i>App</i> * ¹ , <i>MAPT</i> * ⁴	<i>App</i> : KM670/671NL, I716F, <i>MAPT</i> : No mutation	RBRC (RBRC10042)	Saito et al., 2019
<i>App</i> ^{NL-G-F} KI/ <i>hMAPT</i> KI	<i>App</i> * ¹ , <i>MAPT</i> * ⁴	<i>App</i> : KM670/671NL E693G, I716F, <i>MAPT</i> : No mutation	RBRC (RBRC10043)	Saito et al., 2019
<i>hMAPT</i> ^{P301L} KI	<i>MAPT</i> * ⁴	P301L	Available* ⁷	Watanura et al., 2021b
<i>hMAPT</i> ^{P301S} KI	<i>MAPT</i> * ⁴	P301S	Available* ⁷	Watanura et al., 2021b
<i>hMAPT</i> ^{P301V} KI	<i>MAPT</i> * ⁴	P301V	Available* ⁷	Watanura et al., 2021b
<i>hMAPT</i> ^{Intron10+3G>A} KI	<i>MAPT</i> * ⁴	Intron10 + 3 G > A	Available* ⁷	Watanura et al., 2021b
<i>hMAPT</i> ^{P301L;Intron10+3G>A} KI	<i>MAPT</i> * ⁴	P301L; Intron10 + 3 G > A	Available* ⁷	Watanura et al., 2021b
<i>hMAPT</i> ^{P301S;Intron10+3G>A} KI	<i>MAPT</i> * ⁴	P301S; Intron10 + 3 G > A	Available* ⁷	Watanura et al., 2021b
<i>hMAPT</i> ^{S305N;Intron10+3G>A} KI	<i>MAPT</i> * ⁴	S305N; Intron10 + 3 G > A	Available* ⁷	Watanura et al., 2021b

*¹Knock-in of APP sequence (from intron 15 to intron 17) including a humanized Aβ region.

*²The mutation was introduced into the murine *Psen1* gene by using Base Editor (BE) or Target-AID.

*³The mutation was introduced into the murine *Psen1* gene by using VQR-BE.

*⁴Replaced the entire genomic sequence of the murine *Mapt* gene (from exon 1 to exon 14) with the human *MAPT* gene from the ATG codon of exon 1 to the 3'-UTR.

*⁵As of September 30, 2021.

*⁶RIKEN BioResource Research Center (<https://web.brc.riken.jp/en/>).

*⁷All strains are available through TCS (takaomi.saido@riken.jp).

Maintaining Mouse Lines on a Clean C57BL6/J Background

In most cases, the knock-in mice are used in a homozygous state to accelerate the generation of pathological and behavioral phenotypes. However, the number of recessive mutations increases over time if the breeding of mice is maintained in this way. It is necessary therefore to back-cross heterozygous mutant mice with the wild-type C57BL6/J mice to remove these extraneous mutations, preferentially for 5–10 generations at an interval of 5–10 generations. Those groups dealing with poor reproductive output of mice due to their extremely inbred nature can contact RIKEN BioResource Research Center (email: animal.brc@riken.jp), a national mouse repository of Japan (Mizuno-Iijima et al., 2021), to renew their strains.

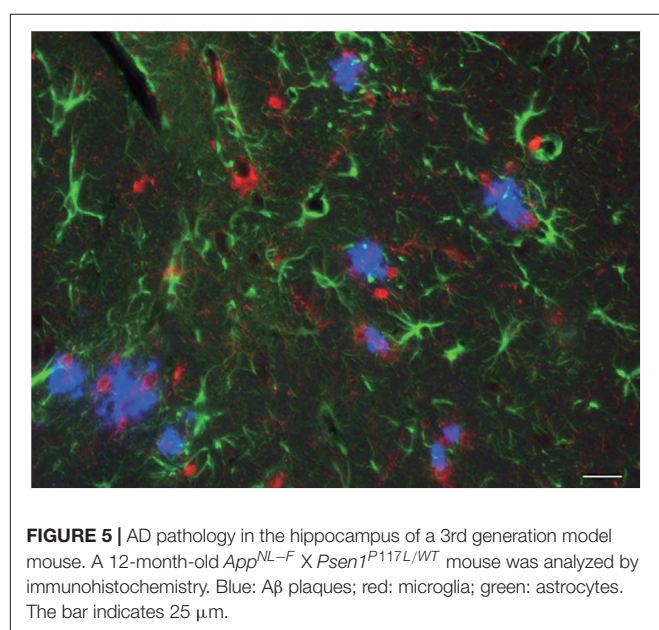
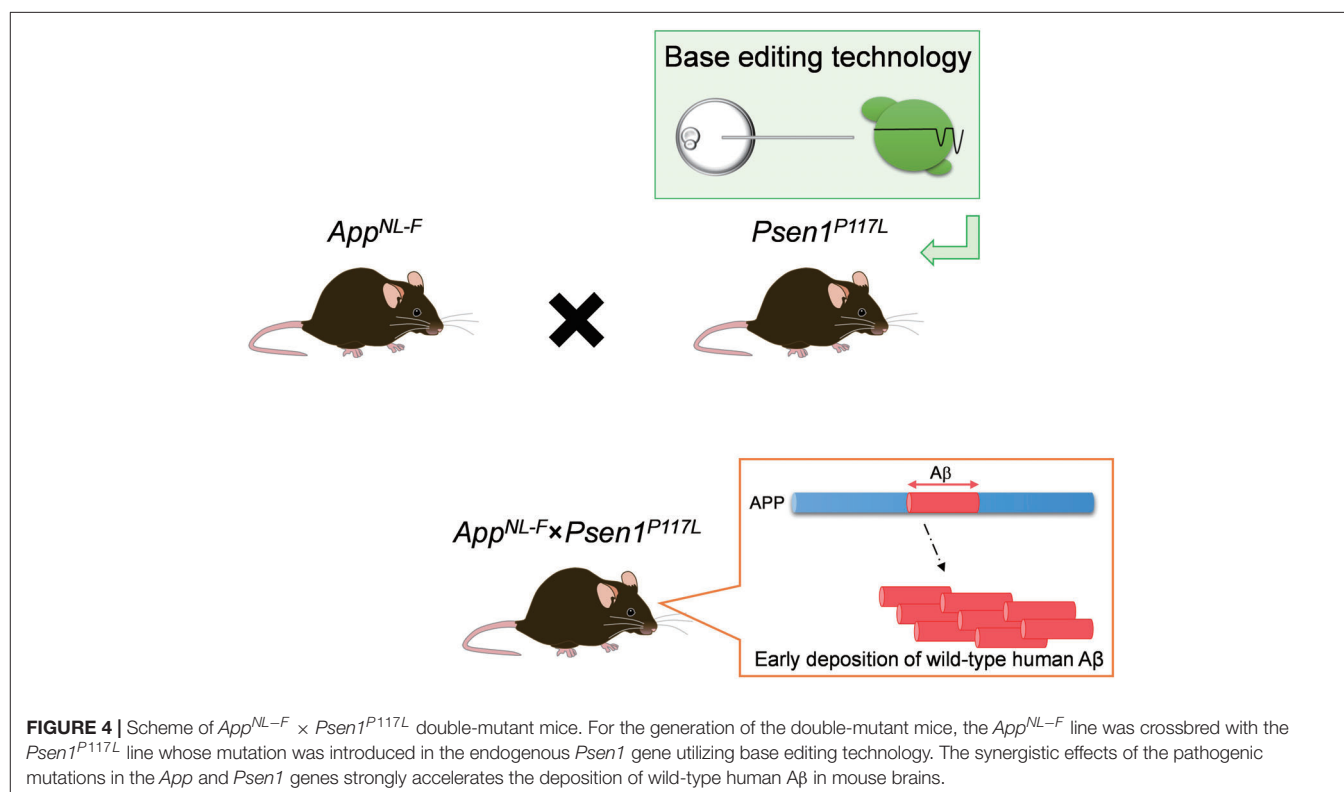
Choosing Appropriate Anti-Aβ Antibodies

It was previously indicated that some anti-Aβ antibodies are inappropriate for biochemically and pathologically detecting

the Arctic Aβ produced by *App*^{NL-G-F} mice (Saito et al., 2014; **Figure 7**). Note that the *App*^{NL-G-F} is the most frequently used model because it recapitulates Aβ pathology and neuroinflammation much faster than other lines. This applies to all other transgenic and knock-in mice that carry intra-Aβ mutations, including the Arctic and Dutch mutations (Van Broeckhoven et al., 1990; Li et al., 2014). In contrast, there is no restriction regarding use of antibodies for the *App*^{NL-F} X *Psen1*^{P117L} mice.

Preclinical Nature of Behaviors

In our experience, the most sensitive and reproducible test involves contextual fear conditioning, although its irreversible nature can be problematic. In a more complex sense, the application of a multi-task paradigm such as *IntelliCage* (NewBehavior AG, Zurich, Switzerland) would be more informative (Codita et al., 2010; Masuda et al., 2016). We recently showed that the mouse genotypes can be predicted from their behavioral parameters by machine learning (Sutoko et al., 2021). It should be emphasized that the *App* knock-in



mice are models of preclinical AD because the *App* knock-in mice, like all the APP and APP/PS1 transgenic mice, do not recapitulate tau pathology alone (Sasaguri et al., 2017). Consistently, we observe only mild cognitive decline in these mice. In contrast, the *App* knock-in mice crossbred with mutant *MAPT* knock-in mice exhibited accelerated tau pathology (Table 2).

A NEW MODEL OF CEREBRAL AMYLOID ANGIOPATHY

Most AD patients exhibit parenchymal and vascular $A\beta$ deposition in the brains, and both pathologies seem to be driven by impaired $A\beta$ clearance within the interstitial fluid and perivascular drainage pathways (Greenberg et al., 2020). Iliff et al. (2012) injected fluorescent tracers into Tie2-GFP:NG2-DsRed double reporter mice, which express GFP in all cerebral blood vessels and DsRed in perivascular cells, and successfully observed glymphatic pathway; subarachnoid CSF influx into the brain parenchyma and bulk ISF solute clearance from the parenchyma within the perivascular spaces. Importantly, in AD model mice, glymphatic CSF influx is reduced and the clearance of $A\beta$ is severely impaired (Peng et al., 2016). Impaired glymphatic pathway may contribute to the deposition of $A\beta$ in the blood vessels of the brain, cerebral amyloid angiopathy (CAA). Although CAA is profoundly observed in most AD patients (Brenowitz et al., 2015), limited model mice, such as those with Dutch/Iowa mutation, exhibit apparent CAA, thus making it difficult to determine how CAA contributes to the pathogenesis of sporadic AD. Notably, human vascular endothelial cells express significant level of APP770 and human plasma contains ~ 100 ng/ml of sAPP770 (Kitazume et al., 2010). Since peripheral blood cells other than platelet do not express APP, and platelets release sAPP770 upon their activation (Miura et al., 2020), it is considered that plasma sAPP770 is mostly derived from endothelial APP770. Because in rodents

TABLE 3 | Characteristics of the *App* knock-in mouse lines.

	Stain	Gene mutations		Genetic background	A β plaques (first appearance)	Tangles	Neuronal loss	Cognitive impairment	Strengths	Weaknesses
		<i>App</i>	<i>Psen1</i>							
Single <i>App</i> knock-in	<i>App</i> ^{huAβ}	Humanized A β	–	C57BL/6J	–	NR	NR	NR	A control for other models	–
	<i>App</i> ^{NL}	Humanized A β KM670/671NL	–	C57BL/6J	–	–	–	–	A control for other models	No amyloid pathology No cognitive deficits Increased CTF- β
	<i>App</i> ^{NL–F}	Humanized A β KM670/671NL I716F	–	C57BL/6J	6 months	–	–	18 months	Deposition of wild type human A β	Long time required for amyloid pathology and cognitive deficits Increased CTF- β
	<i>App</i> ^{NL–G–F}	Humanized A β KM670/671NL E693G I716F	–	C57BL/6J	2 months	–	–	6 months	Early appearance of amyloid pathology	The Arctic mutation inside the A β sequence Increased CTF- β
	<i>App</i> ^{G–F}	Humanized A β E693G I716F	–	C57BL/6J	4 months	NR	NR	NR	Absence of the Swedish mutation No increase of CTF- β	The Arctic mutation inside the A β sequence
<i>App</i> and <i>Psen1</i> double mutant	<i>App</i> ^{NL–F} <i>Psen1</i> ^{P117L}	Humanized A β KM670/671NL I716F	P117L	C57BL/6J	3 months	NR	NR	NR	Early appearance of amyloid pathology Deposition of wild type human A β	Mutations in both <i>App</i> and <i>Psen1</i> genes

The Swedish mutations; KM670/671NL.

The Iberian/Beyreuther mutation; I716F.

The Arctic mutation; E693G.

NR denotes data not reported.

plasma sAPP is a markedly lower (~100 pg/ml) than that of humans (Kitazume et al., 2012), it's possible that low level of endothelial APP expression in mice could be one of the reasons that *App* knock-in mice exhibit mild CAA pathology. To overcome this, a mouse line that specifically expresses human APP770 in endothelial cells has just been generated (unpublished). In brief, floxed hAPP770NL mice under the CMV early enhancer/chicken β -actin promoter were first generated. These mice were then crossed with Tie2-Cre mice, in which the Tie2 promoter directs the expression of Cre recombinase in the endothelial cells to obtain double transgenic (Tg) mice.

App knock-in mouse models were previously produced by Li et al. (2014) who used multiple pathogenic mutations. These mice carry the Swedish (K670N/M671L), Dutch (E693Q), and London (V717I) mutations with the humanized A β sequence. The Dutch mutation results in an intensive CAA pathology in humans, thereby causing brain hemorrhage and early mortality (Levy et al., 1990; Van Broeckhoven et al., 1990). This mutation is therefore not specifically responsible for causing FAD. These mice did not develop prominent A β deposits over their lifespan, but when they were crossbred with *Psen1*^{M146V} knock-in mice, an age-dependent deposition of A β was seen in the resultant double

knock-in mice. The deposition of A β was detected not only in the parenchyma of the cerebral cortex but also in the cerebral vasculature, similar to that seen in CAA in humans. Double knock-in mice that did not have the Dutch mutation exhibited virtually no vascular pathology. In this way, if the authors had used the Beyreuther/Iberian or Austrian mutation instead of the London mutation in the mouse *App* gene then they probably would not have had to introduce the *Psen1* knock-in mice. Knock-in mice harboring the Dutch mutation can still serve as relevant models for CAA; however they may not be appropriate for examining the effect of immunotherapy on CAA because the Dutch mutation is present in the middle of the A β sequence.

NEPRILYSIN-SENSITIVE AMYLOIDOGENIC A β AS A PROBABLE CAUSE FOR SPORADIC ALZHEIMER'S DISEASE

NEP and insulin-degrading enzyme (IDE) are considered the two major catabolic enzymes that degrade A β (Qiu et al., 1998; Iwata et al., 2000, 2001; Farris et al., 2003; Leissring et al., 2003). NEP is capable of degrading both soluble and insoluble A β

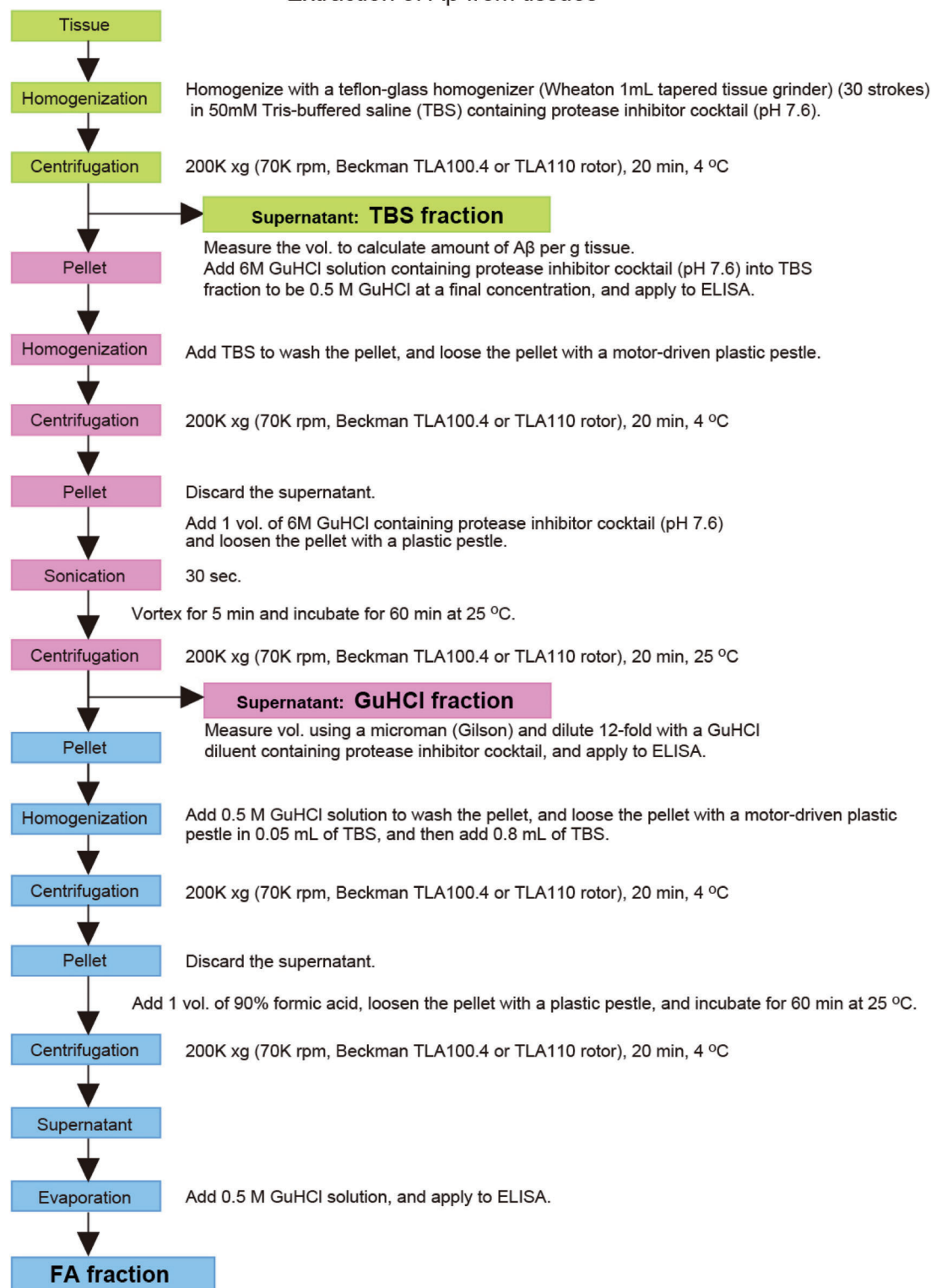
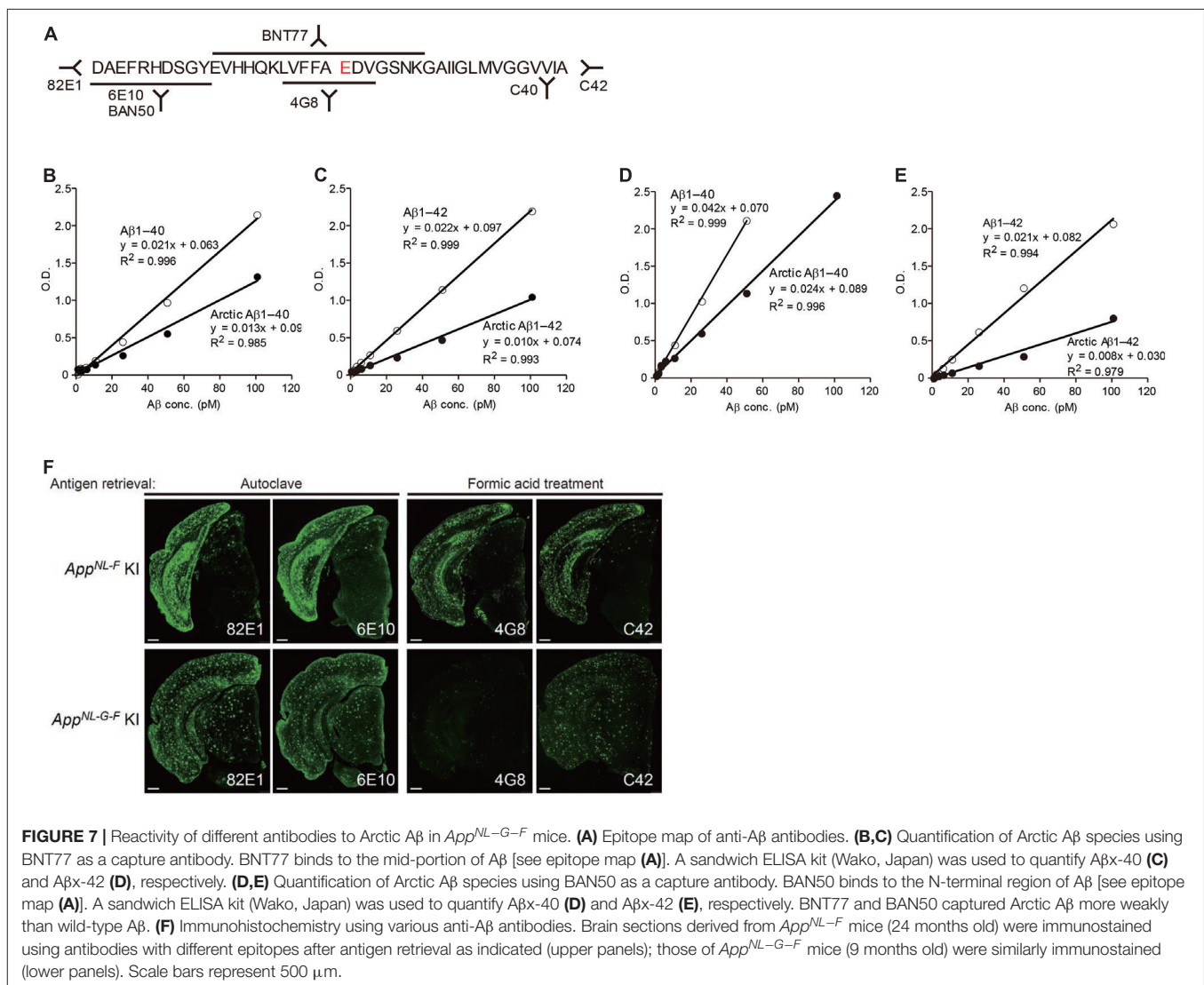
Extraction of A β from tissues

FIGURE 6 | Outlined protocols for extraction and quantification of A β from tissues. See text for detailed explanation.



(Iwata et al., 2000, 2001; Huang et al., 2006), but it is not clearly shown whether endogenous IDE could degrade insoluble Aβ in the mouse brains (Farris et al., 2003), rather IDE appears to be involved in metabolism of AICD. Unfortunately, their roles in Aβ metabolism *in vivo* have never been compared in an impartial and side-by-side manner. Once double mutants crossbred single *App* knock-in mice with NEP (*Mme*) KO mice and with IDE (*Ide*) KO mice were analyzed in detail for their biochemical properties and Aβ pathology properties, it would be clear their distinct roles in APP metabolism and the AD pathogenesis.

Further to the above, a deficiency of NEP had no significant impact on the levels of various neuropeptides (Sasaguri et al., 2018) as well as enkephalins (Saria et al., 1997) that are well known to be *in vitro* substrates for NEP (Turner et al., 1996, 2000, 2001; Turner and Nalivaeva, 2006) in the cerebral cortex and hippocampal formation of mice, presumably because NEP is mainly expressed in secretory vesicles and on the presynaptic membranes of excitatory neurons (Iwata et al., 2002, 2004, 2013), while most if not all neuropeptides are secreted from

inhibitory neurons. This argues against the concern that NEP up-regulation for the treatment of preclinical AD would reduce the levels of these neuropeptides. These findings also indicate that NEP relatively selectively degrades Aβ in the brain. Whereas familial AD (FAD) is unambiguously caused by an increased anabolism of Aβ, and of Aβ₄₂ and Aβ₄₃ in particular (Selkoe and Hardy, 2016), the anabolism of Aβ appears unaffected prior to its deposition in the brain that subsequently leads to the onset of sporadic AD (SAD). These observations suggest that NEP-sensitive amyloidogenic Aβ likely plays a primary pathogenic role in the etiology of SAD. Our findings are consistent with the aging-dependent decline of NEP expression in the human brain and with recent genome-wide association studies (GWAS) indicating that variants of the gene encoding NEP (*MME*) are associated with the risk of SAD development (Bellenguez et al., 2020). Taken together, our results imply that the aging-associated decrease in NEP expression is a primary cause of SAD and could thus be a target for the treatment of preclinical AD once other factors such as apolipoprotein E genotypes have also been considered.

REGULATION OF A β METABOLISM VIA SOMATOSTATIN RECEPTOR SUBTYPES THROUGH MODULATION OF NEP ACTIVITY

Since NEP is a major A β -degrading enzyme and it is downregulated upon aging, its decreased levels in the brain will most likely lead to increased A β levels (Yasojima et al., 2001; Carpentier et al., 2002; Iwata et al., 2002; Maruyama et al., 2005; Hellström-Lindh et al., 2008). NEP is regulated by the neuropeptide somatostatin (Saito et al., 2005). Somatostatin, which binds to somatostatin receptors, is also decreased upon aging and in AD possibly due to loss of somatostatin-positive interneurons (Davies et al., 1980; Beal et al., 1985; Bergström et al., 1991; Hayashi et al., 1997; van de Nes et al., 2002; Lu et al., 2004; Gahete et al., 2010). Somatostatin, which was first identified to regulate secretion of growth hormone from pituitary, has been traditionally abbreviated as SRIF (somatotropin release-inhibiting factor) (Møller et al., 2003), so we will keep to this nomenclature in this review. SST₁, SST₂, SST₃, SST₄, and SST₅ are used to express somatostatin receptor subtypes 1–5. Interestingly, mutations in SRIF are linked to AD (Vepsäläinen et al., 2007). By using a combination of *in vitro* and *in vivo* approaches to identify the subtype specificity of the five SSTs expressed in the brain and considered to be involved in the regulation of NEP. We would like to emphasize that it is necessary to use a co-culture system of primary neurons from the cortex, hippocampus, and striatum for *in vitro* experiments (Kakiya et al., 2012; Nilsson et al., 2020; Watamura et al., 2021a).

Using a battery of *Sst* double knockout (dKO) mice, we found that NEP is regulated by SST₁ and SST₄ in a redundant manner. *Sst*₁ and *Sst*₄ dKO mice exhibit a specific decrease of presynaptic NEP in the Lacunosum molecular layer. Moreover, a genetic deficiency of *Sst*₁ and *Sst*₄ in the *App* knock-in mice aggravated the A β pathology in the hippocampus. As a first proof of concept towards an A β -lowering strategy involving NEP, a treatment with an agonist selective for SST₁ and SST₄ could ameliorates the A β pathology and improves cognitive outcomes in the *App* knock-in AD mouse model as schematized in **Figure 8** (Nilsson et al., 2020). These results indicate that a combination of SST₁ and SST₄ homodimers or the SST₁ and SST₄ heterodimer may become a target for pharmaceutical intervention to treat preclinical AD. Of note, the expression of SRIF in human brain declines with aging and in AD (Davies et al., 1980; Lu et al., 2004) and may causally contribute to AD pathogenesis *via* reduction of NEP activity/expression.

SRIF-EVOKED A β CATABOLISM IN THE BRAIN: MECHANISTIC INVOLVEMENT OF THE α -ENDOSULFINE-K_{ATP} CHANNEL PATHWAY

Although SRIF is known to regulate A β catabolism by enhancing NEP-catalyzed proteolytic degradation, the mechanism by which SRIF actually regulates NEP activity is yet to be fully

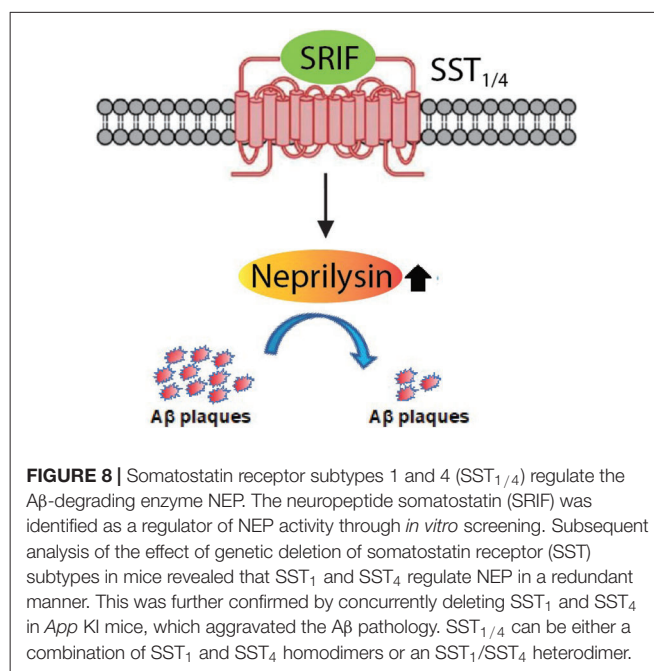


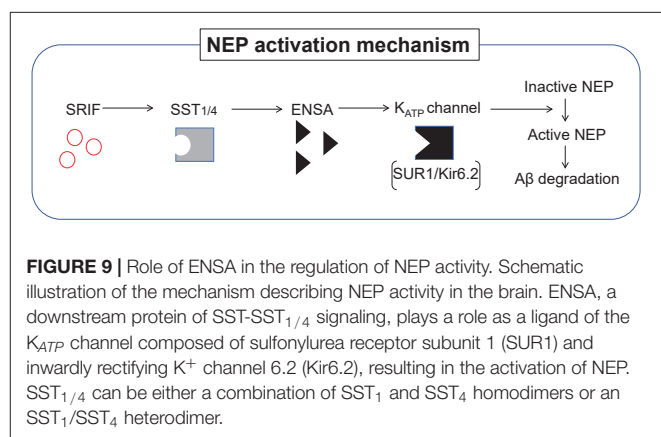
FIGURE 8 | Somatostatin receptor subtypes 1 and 4 (SST_{1/4}) regulate the A β -degrading enzyme NEP. The neuropeptide somatostatin (SRIF) was identified as a regulator of NEP activity through *in vitro* screening. Subsequent analysis of the effect of genetic deletion of somatostatin receptor (SST) subtypes in mice revealed that SST₁ and SST₄ regulate NEP in a redundant manner. This was further confirmed by concurrently deleting SST₁ and SST₄ in *App* KI mice, which aggravated the A β pathology. SST_{1/4} can be either a combination of SST₁ and SST₄ homodimers or an SST₁/SST₄ heterodimer.

elucidated. Proteomic analyses enabled α -endosulfine (ENSA), an endogenous ligand of the ATP-sensitive potassium (K_{ATP}) channel, to be identified as a negative regulator of NEP downstream of SRIF signaling (Watamura et al., 2021a). The expression of ENSA is significantly increased in AD mouse models and in patients with AD. In addition, NEP directly contributes to the degradation of ENSA, suggesting a substrate-dependent feedback loop regulating NEP activity.

It was also discovered the specific K_{ATP} channel subtype [sulfonylurea receptor subunit 1 (SUR1) and inwardly rectifying K⁺ channel 6.2 (Kir6.2)] that modulates NEP activity, resulting in altered A β levels in the brain. Pharmacological intervention targeting this particular K_{ATP} channel by diazoxide attenuated A β deposition, with impaired memory function rescued *via* the NEP activation in our AD mouse model. These findings provide a mechanism explaining the molecular link between K_{ATP} channels and NEP activation. They also provide new insights into how ENSA and the K_{ATP} channel could profile as a new therapeutic target for lowering A β and thus provide an alternative strategy to prevent AD. **Figure 9** summarizes the NEP activation mechanism that involves SRIF receptor subtypes, ENSA and K_{ATP} channel involvement.

HUMANIZATION OF THE ENTIRE MURINE *Mapt* GENE TO GENERATE hMAPT KNOCK-IN MICE

To date, most if not all, mouse models of tauopathy have been unable to recapitulate the tau pathology without overexpressing mutant human tau protein. As a novel *in vivo* platform for studying human tauopathy, human *MAPT* knock-in mice have been developed in which the entire *Mapt* gene including all exons

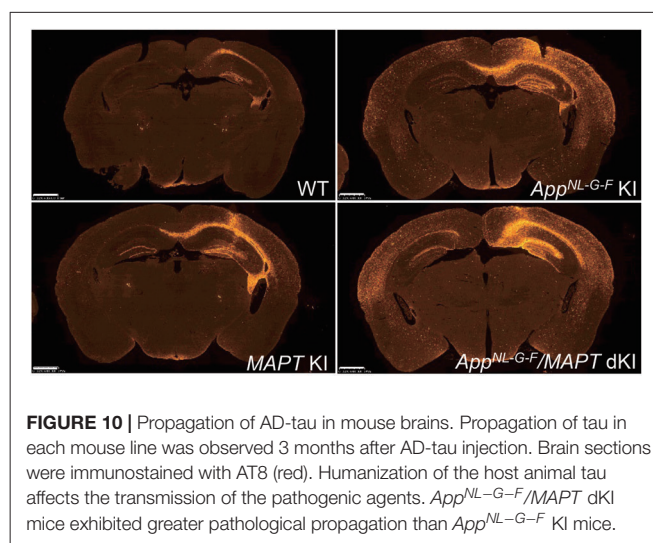


and introns are humanized (Hashimoto et al., 2019). In each strain, the *MAPT* and *Mapt* genes encoded human and murine tau proteins, respectively. This was done by crossing *MAPT* knock-in mice with single *App* knock-in mice in order to study the role of the Aβ-tau axis in the etiology of AD. The double knock-in mice exhibited a more pronounced tau phosphorylation status than single *MAPT* knock-in mice but lacked evidence of tau pathology and neurodegeneration (in a manner similar to that of single *App* knock-in mice) even after waiting until the mice were 24 months old.

In both the absence and presence of Aβ amyloidosis, the tau humanization has been found to significantly accelerate the propagation of AD brain-derived pathological tau (Figure 10; Saito et al., 2019). Tau accumulation was intensified in the latter case and closely associated with dystrophic neurites, consistently showing that Aβ amyloidosis affects tau pathology. These results indicated that pathological human tau interacted better with human tau than with murine tau, and suggest the presence of a species-defined preference between the pathogenic proteins. The *MAPT* knock-in mice also facilitate the investigation of behavioral properties and of human tau characteristics in living animal models. In addition, mutant *MAPT* knock-in mice carrying various pathogenic mutations have been generated (Table 2). These mice exhibit aging-dependent tau aggregation and cognitive impairment in a manner accelerated by Aβ pathology and are being provided to the research community upon request.

THE ROLE OF CAPON IN TAU PATHOLOGY-MEDIATED TOXICITY

Pathological tau causes synaptic dysfunction and loss of synapses. One of promising molecules that mediates tau pathology-induced neurotoxicity is *N*-methyl-D-aspartate receptor (NMDAR). For example, tau accumulation disturbs synaptic plasticity through JAK2/STAT1-mediated suppression of NMDAR (Li et al., 2019). Phosphorylation of tau at Tyr18, which is mediated by the tyrosine kinase Fyn, enhances NMDAR-dependent excitotoxicity (Guo et al., 2020). Several reports indicated that glutamate-induced excitotoxicity was prevented by downmodulation of tau

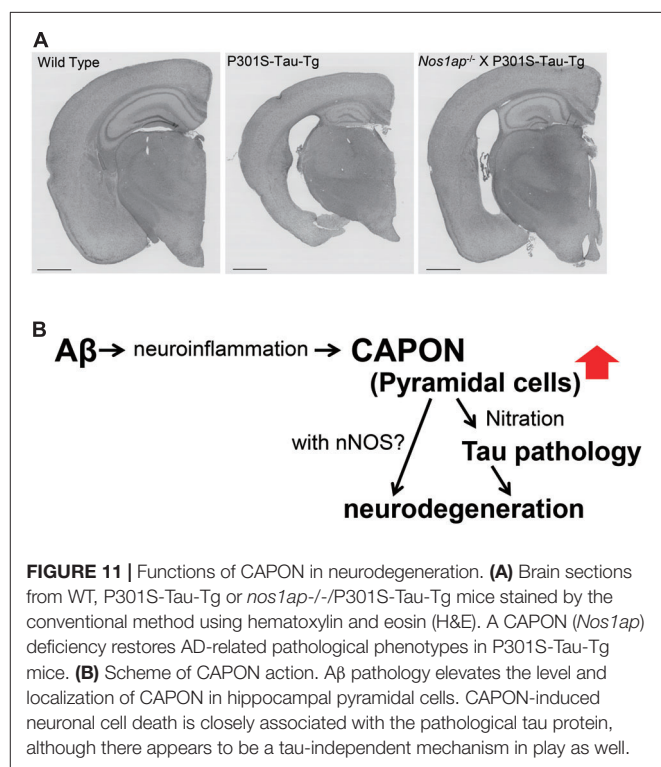


(Roberson et al., 2007) (Ittner et al., 2010). We also previously identified a NMDAR-related molecule as a tau binding protein which is involved in tau pathology-induced neurodegeneration.

To elucidate key molecules underlying tau accumulation-induced neurodegeneration, a comprehensive screening of tau-interacting proteins (tau interactome) was constructed. Tau-binding proteins were isolated by immunoprecipitation-LC-MS/MS (IP-MS) using a Flag-tag antibody and wild-type tau Tg (wtau-Tg) mice, which is expressing human tau tagged with a flag epitope (Kimura et al., 2007). Considering that tau is a microtubule-binding protein, we validated the methods used to generate the tau interactome by identifying the tubulin beta-4A chain as one of the tau-binding proteins.

Of the many proteins identified in the tau interactome, we focused on carboxy-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON) (Hashimoto et al., 2019), which is an adaptor protein of neuronal nitric oxide synthase (nNOS). CAPON acts as an enzyme for the production of nitric oxide (NO) and is involved in NMDAR-mediated excitotoxicity (Jaffrey et al., 1998). It is thought to recruit substrates to nNOS and facilitate their NO-mediated modification through protein-protein interactions (Jaffrey et al., 1998). The presence of CAPON polymorphisms associated with schizophrenia and other psychiatric disorders has been reported in several studies (Brzustowicz, 2008; Freudenberg et al., 2015). Moreover, CAPON was shown to positively regulate spine density (Richier et al., 2010) and to regulate neuronal cell death downstream of the NMDAR (Li et al., 2013). These findings suggest that CAPON contributes to neurotransmission and neuronal excitotoxicity. In addition, one report showed that CAPON is upregulated in CA1 pyramidal cells in the AD brain (Hashimoto et al., 2012), implying that CAPON may play an important role in the pathogenesis of AD. The mechanism(s) underlying these effects nevertheless remain(s) unknown.

To further elucidate the effects of CAPON on AD pathology, we introduced CAPON cDNA into the brains of *App*^{NL-G-F} and *App*^{NL-G-F} X *MAPT* (hTau) double-KI mice using a



newly developed adeno-associated virus (AAV)-mediated approach. We analyzed the effects of human tau protein as it is known that the hTau-KI mouse expresses an endogenous level of WT human tau. These experiments revealed that CAPON expression facilitates hippocampal atrophy in conjunction with neuronal cell death, and that a deficiency of CAPON in the P301S-Tau-Tg tauopathy mouse model suppressed tau pathology and neurodegeneration (**Figure 11**). From our results, an intervention in the interaction between CAPON-tau or CAPON-nNOS could be a new approach for the treatment of AD.

GENERATION OF NON-HUMAN PRIMATE MODELS OF FAMILIAL ALZHEIMER'S DISEASE

Common marmosets (marmosets, *Callithrix jacchus*) are small non-human primates that belong to the New World Primates (**Figure 12**; Mansfield, 2003). They have been increasingly utilized in neuroscience because of advantages that were observed over other research primates (Okano, 2021; Park and Sasaki, 2021). Marmosets possess physiological functions, brain structures and complex cognitive/social behaviors similar to those of humans; they communicate mainly *via* visual and auditory measures. In association with AD research, the amino acid sequence of A β in marmosets is identical to that of humans, with aged wild-type marmosets starting to accumulate A β from 7 years of age or even earlier (Geula et al., 2002; Rodriguez-Callejas et al., 2016). In addition,



FIGURE 12 | Photograph of common marmosets (*Callithrix jacchus*). The photo shows members of captive common marmoset family. Their small body size, fecundity, and high cognitive functions are a suitable model for neuroscience. The photograph of marmosets was taken by WK at CIEA.

adolescent marmosets exhibit tau hyperphosphorylation, but not neurofibrillary tangle formation, in the brain that increases with aging (Rodriguez-Callejas et al., 2016). Their life spans in captivity are as long as 10–15 years, making them suitable for age-related research (Tardif et al., 2011). Their immune systems and metabolic functions resemble those of humans (t Hart and Massacesi, 2009; Tardif et al., 2011) and thus may affect the pathogenic processes related to AD (Ennerfelt and Lukens, 2020; Kellar and Craft, 2020; Rosario et al., 2020). Because sleep disorder is an early clinical symptom of AD (Pyun et al., 2019), it is noteworthy that marmosets share with humans the sleep phases composed of rapid eye movement (REM) and non-REM cycles (Crofts et al., 2001). Among various non-human primate species, the marmoset seems most applicable to genetic manipulation, i.e., generation of designed mutants, for which their high reproductive efficacy is advantageous (Sasaki et al., 2009; Sato et al., 2016; Park and Sasaki, 2021). Furthermore, fecundity characteristics of marmosets, such as a short period of sexual maturity, multiple births, and short gestation interval, are suitable for producing genetically modified disease models (Tardif et al., 2003).

Majority of FAD-causing mutations reside in the *PSEN1* gene (Searce-Levie et al., 2020). Typically, deletion mutations in exon 9 (Crook et al., 1998; Prihar et al., 1999; Smith et al., 2001; Dumanchin et al., 2006; Le Guennec et al., 2017) or point mutations at the 3' splice site (acceptor site) of exon 9 in the *PSEN1* gene cause dominantly inherited FAD. The point mutations instigate exon 9 elimination and S290C modification in the corresponding mRNA at the junction sites of exons 8 and 10 *via* the conversion of alternative splicing (Hutton et al., 1996; Kwok et al., 1997; Steiner et al., 1999; Brooks et al., 2003; Blauwendraat et al., 2016). Thus, generation of a marmoset model of AD is set out in which exon 9 of the *PSEN1* gene product is deleted using gene-editing technologies to produce AD marmoset

models. Since TALEN exhibited high genome-editing efficacy, generates few off-target effects, and produces little mosaicism, the TALEN would be a suitable tool for producing exon 9 deletion in the *PSEN1* gene (Sato et al., 2016; Zhang et al., 2019). Although it is a non-peer review data, the exon 9 deletion in the *PSEN1* gene that is an AD causing mutation has been successfully introduced into non-human primates by TALEN (Sato et al., 2020).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

Part of the work described was supported by the following research grants: JSPS KAKENHI Grant Number JP18K07402

REFERENCES

- Barrett, T., Stangis, K. A., Saito, T., Saido, T., and Park, K. H. J. (2021). Neuronal cell cycle re-entry enhances neuropathological features in AppNLf knock-in mice. *J. Alzheimers Dis.* 82, 1683–1702. doi: 10.3233/jad-210091
- Basun, H., Bogdanovic, N., Ingelsson, M., Almkvist, O., Naslund, J., Axelman, K., et al. (2008). Clinical and neuropathological features of the arctic APP gene mutation causing early-onset Alzheimer disease. *Arch. Neurol.* 65, 499–505. doi: 10.1001/archneur.65.4.499
- Beal, M. F., Mazurek, M. F., Tran, V. T., Chattha, G., Bird, E. D., and Martin, J. B. (1985). Reduced numbers of somatostatin receptors in the cerebral cortex in Alzheimer's disease. *Science* 229, 289–291. doi: 10.1126/science.2861661
- Bellenguez, C., Küçükali, F., Jansen, I., Andrade, V., Moreno-Grau, S., Amin, N., et al. (2020). New insights on the genetic etiology of Alzheimer's and related dementia. *medRxiv [preprint]* doi: 10.1101/2020.10.01.20200659
- Bergström, L., Garlind, A., Nilsson, L., Alafuzoff, I., Fowler, C. J., Winblad, B., et al. (1991). Regional distribution of somatostatin receptor binding and modulation of adenylyl cyclase activity in Alzheimer's disease brain. *J. Neurol. Sci.* 105, 225–233. doi: 10.1016/0022-510x(91)90149-2
- Blauwendraat, C., Wilke, C., Jansen, I. E., Schulte, C., Simon-Sanchez, J., Metzger, F. G., et al. (2016). Pilot whole-exome sequencing of a German early-onset Alzheimer's disease cohort reveals a substantial frequency of PSEN2 variants. *Neurobiol. Aging* 37, 208.e11–208.e17. doi: 10.1016/j.neurobiolaging.2015.09.016
- Brenowitz, W. D., Nelson, P. T., Besser, L. M., Heller, K. B., and Kukull, W. A. (2015). Cerebral amyloid angiopathy and its co-occurrence with Alzheimer's disease and other cerebrovascular neuropathologic changes. *Neurobiol. Aging* 36, 2702–2708. doi: 10.1016/j.neurobiolaging.2015.06.028
- Brooks, W. S., Kwok, J. B., Kril, J. J., Broe, G. A., Blumbers, P. C., Tannenberg, A. E., et al. (2003). Alzheimer's disease with spastic paraparesis and 'cotton wool' plaques: two pedigrees with PS-1 exon 9 deletions. *Brain* 126, 783–791. doi: 10.1093/brain/awg084
- Brzustowicz, L. M. (2008). NOS1AP in schizophrenia. *Curr. Psychiatry Rep.* 10, 158–163.
- Carpentier, M., Robitaille, Y., DesGroseillers, L., Boileau, G., and Marcinkiewicz, M. (2002). Declining expression of neprilysin in Alzheimer disease vasculature: possible involvement in cerebral amyloid angiopathy. *J. Neuropathol. Exp. Neurol.* 61, 849–856. doi: 10.1093/jnen/61.10.849
- Chen, W. T., Lu, A., Craessaerts, K., Pavie, B., Sala Frigerio, C., Corthout, N., et al. (2020). Spatial transcriptomics and *in situ* sequencing to study alzheimer's disease. *Cell* 182, 976–991.e19. doi: 10.1016/j.cell.2020.06.038
- (HS); JSPS KAKENHI Grant Numbers JP15K19036 and JP19K16271 and RIKEN Special Postdoctoral Research program (SH); JSPS KAKENHI Grant Number JP21K15378 (NW); AMED Grant Number JP19dm0207065 (ES), AMED Grant Number JP18am0101036 (SK); The Swedish Research Council 2018-02843 (BW); Hållsten Research Foundation (PN); JSPS KAKENHI Grant Number JP20H03564/AMED Grant Numbers JP21gm1210010s0102 and JP21dk0207050h001/JST [Moonshot R&D] Grant Number JPMJMS2024/Grant-in-aid for Research in Nagoya City University Grant Number 2021101/the Hori Sciences and Arts Foundation, and Toyoaki Scholarship Foundation (TS); AMED Grant Numbers JP16dm0107068h, JP17dm0107068h, JP18dm0107068h (NI and TS); AMED Grant Number JP15dm0207001 and RIKEN Aging Project (TCS).
- ## ACKNOWLEDGMENTS
- We thank Yukiko Nagai-Watanabe for secretarial assistance.
- Chiasseu, M., Fesharaki-Zadeh, A., Saito, T., Saido, T. C., and Strittmatter, S. M. (2020). Gene-environment interaction promotes Alzheimer's risk as revealed by synergy of repeated mild traumatic brain injury and mouse App knock-in. *Neurobiol. Dis.* 145:105059. doi: 10.1016/j.nbd.2020.105059
- Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., et al. (1992). Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature* 360, 672–674. doi: 10.1038/360672a0
- Codita, A., Gumucio, A., Lannfelt, L., Gellerfors, P., Winblad, B., Mohammed, A. H., et al. (2010). Impaired behavior of female tg-ArcSwe APP mice in the IntelliCage: a longitudinal study. *Behav. Brain Res.* 215, 83–94. doi: 10.1016/j.bbr.2010.06.034
- Crofts, H. S., Wilson, S., Muggleton, N. G., Nutt, D. J., Scott, E. A., and Pearce, P. C. (2001). Investigation of the sleep electrocorticogram of the common marmoset (*Callithrix jacchus*) using radiotelemetry. *Clin. Neurophysiol.* 112, 2265–2273. doi: 10.1016/s1388-2457(01)00699-x
- Crook, R., Verkkoniemi, A., Perez-Tur, J., Mehta, N., Baker, M., Houlden, H., et al. (1998). A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. *Nat. Med.* 4, 452–455. doi: 10.1038/nm0498-452
- Davies, P., Katzman, R., and Terry, R. D. (1980). Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia. *Nature* 288, 279–280. doi: 10.1038/288279a0
- Dumanchin, C., Tournier, I., Martin, C., Didic, M., Belliard, S., Carlander, B., et al. (2006). Biological effects of four PSEN1 gene mutations causing Alzheimer disease with spastic paraparesis and cotton wool plaques. *Hum. Mutat.* 27:1063. doi: 10.1002/humu.9458
- Ennerfelt, H. E., and Lukens, J. R. (2020). The role of innate immunity in Alzheimer's disease. *Immunol. Rev.* 297, 225–246. doi: 10.1111/imr.12896
- Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E. A., Frosch, M. P., et al. (2003). Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain *in vivo*. *Proc. Natl. Acad. Sci. U S A.* 100, 4162–4167. doi: 10.1073/pnas.0230450100
- Freudenberger, F., Alttou, A., and Reif, A. (2015). Neuronal nitric oxide synthase (NOS1) and its adaptor, NOS1AP, as a genetic risk factors for psychiatric disorders. *Genes Brain Behav.* 14, 46–63. doi: 10.1111/gbb.12193
- Gahete, M. D., Rubio, A., Durán-Prado, M., Avila, J., Luque, R. M., and Castaño, J. P. (2010). Expression of Somatostatin, cortistatin, and their receptors, as well as dopamine receptors, but not of neprilysin, are reduced in the temporal lobe of Alzheimer's disease patients. *J. Alzheimers. Dis.* 20, 465–475. doi: 10.3233/jad-2010-1385

- Gamache, J., Benzow, K., Forster, C., Kemper, L., Hlynialuk, C., Furrow, E., et al. (2019). Factors other than hTau overexpression that contribute to tauopathy-like phenotype in rTg4510 mice. *Nat. Commun.* 10:2479. doi: 10.1038/s41467-019-10428-1
- Geula, C., Nagykeri, N., and Wu, C. K. (2002). Amyloid-beta deposits in the cerebral cortex of the aged common marmoset (*Callithrix jacchus*): incidence and chemical composition. *Acta Neuropathol.* 103, 48–58. doi: 10.1007/s004010100429
- Greenberg, S. M., Bacskai, B. J., Hernandez-Guillamon, M., Pruzin, J., Sperling, R., and van Veluw, S. J. (2020). Cerebral amyloid angiopathy and Alzheimer disease - one peptide, two pathways. *Nat. Rev. Neurol.* 16, 30–42. doi: 10.1038/s41582-019-0281-2
- Guo, T., Zhang, D., Zeng, Y., Huang, T. Y., Xu, H., and Zhao, Y. (2020). Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. *Mol. Neurodegener.* 15:40. doi: 10.1186/s13024-020-00391-7
- Hama, H., Hioki, H., Namiki, K., Hoshida, T., Kurokawa, H., Ishidate, F., et al. (2015). ScaleS: an optical clearing palette for biological imaging. *Nat. Neurosci.* 18, 1518–1529. doi: 10.1038/nn.4107
- Hashimoto, M., Bogdanovic, N., Nakagawa, H., Volkmann, I., Aoki, M., Winblad, B., et al. (2012). Analysis of microdissected neurons by 18O mass spectrometry reveals altered protein expression in Alzheimer's disease. *J. Cell Mol. Med.* 16, 1686–1700. doi: 10.1111/j.1582-4934.2011.01441.x
- Hashimoto, S., Ishii, A., Kamano, N., Watamura, N., Saito, T., Ohshima, T., et al. (2018). Endoplasmic reticulum stress responses in mouse models of Alzheimer's disease: overexpression paradigm versus knockin paradigm. *J. Biol. Chem.* 293, 3118–3125. doi: 10.1074/jbc.M117.811315
- Hashimoto, S., Matsuba, Y., Kamano, N., Mihira, N., Sahara, N., Takano, J., et al. (2019). Tau binding protein CAPON induces tau aggregation and neurodegeneration. *Nat. Commun.* 10:2394. doi: 10.1038/s41467-019-10278-x
- Hayashi, M., Yamashita, A., and Shimizu, K. (1997). Somatostatin and brain-derived neurotrophic factor mRNA expression in the primate brain: decreased levels of mRNAs during aging. *Brain Res.* 749, 283–289. doi: 10.1016/s0006-8993(96)01317-0
- Hellström-Lindh, E., Ravid, R., and Nordberg, A. (2008). Age-dependent decline of neprilysin in Alzheimer's disease and normal brain: inverse correlation with a beta levels. *Neurobiol. Aging* 29, 210–221. doi: 10.1016/j.neurobiolaging.2006.10.010
- Higuchi, M., Iwata, N., Matsuba, Y., Takano, J., Suemoto, T., Maeda, J., et al. (2012). Mechanistic involvement of the calpain-calpastatin system in Alzheimer neuropathology. *FASEB J.* 26, 1204–1217. doi: 10.1096/fj.11-187740
- Hongo, N., Takamura, Y., Nishimaru, H., Matsumoto, J., Tobe, K., Saito, T., et al. (2020). Astaxanthin ameliorated parvalbumin-positive neuron deficits and Alzheimer's disease-related pathological progression in the hippocampus of App(NL-G-F/NL-G-F) Mice. *Front. Pharmacol.* 11:307. doi: 10.3389/fphar.2020.00307
- Huang, S. M., Mouri, A., Kokubo, H., Nakajima, R., Suemoto, T., Higuchi, M., et al. (2006). Neprilysin-sensitive synapse-associated amyloid-beta peptide oligomers impair neuronal plasticity and cognitive function. *J. Biol. Chem.* 281, 17941–17951. doi: 10.1074/jbc.M601372200
- Hutton, M., Busfield, F., Wragg, M., Crook, R., Perez-Tur, J., Clark, R. F., et al. (1996). Complete analysis of the presenilin 1 gene in early onset Alzheimer's disease. *Neuroreport* 7, 801–805. doi: 10.1097/00001756-199602290-00029
- Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β . *Sci. Transl. Med.* 4:147ra111. doi: 10.1126/scitranslmed.3003748
- Ittner, L. M., Ke, Y. D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., et al. (2010). Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell* 142, 387–397. doi: 10.1016/j.cell.2010.06.036
- Iwata, N., Higuchi, M., and Saido, T. C. (2005). Metabolism of amyloid-beta peptide and Alzheimer's disease. *Pharmacol. Ther.* 108, 129–148. doi: 10.1016/j.pharmthera.2005.03.010
- Iwata, N., Mizukami, H., Shirohata, K., Takaki, Y., Muramatsu, S., Lu, B., et al. (2004). Presynaptic localization of neprilysin contributes to efficient clearance of amyloid-beta peptide in mouse brain. *J. Neurosci.* 24, 991–998. doi: 10.1523/jneurosci.4792-03.2004
- Iwata, N., Sekiguchi, M., Hattori, Y., Takahashi, A., Asai, M., Ji, B., et al. (2013). Global brain delivery of neprilysin gene by intravascular administration of AAV vector in mice. *Sci. Rep.* 3:1472. doi: 10.1038/srep01472
- Iwata, N., Takaki, Y., Fukami, S., Tsubuki, S., and Saido, T. C. (2002). Region-specific reduction of a beta-degrading endopeptidase, neprilysin, in mouse hippocampus upon aging. *J. Neurosci. Res.* 70, 493–500. doi: 10.1002/jnr.10390
- Iwata, N., Tsubuki, S., Takaki, Y., Shirohata, K., Lu, B., Gerard, N. P., et al. (2001). Metabolic regulation of brain Abeta by neprilysin. *Science* 292, 1550–1552. doi: 10.1126/science.1059946
- Iwata, N., Tsubuki, S., Takaki, Y., Watanabe, K., Sekiguchi, M., Hosoki, E., et al. (2000). Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat. Med.* 6, 143–150. doi: 10.1038/72237
- Jaffrey, S. R., Snowman, A. M., Eliasson, M. J., Cohen, N. A., and Snyder, S. H. (1998). CAPON: a protein associated with neuronal nitric oxide synthase that regulates its interactions with PSD95. *Neuron* 20, 115–124. doi: 10.1016/s0896-6273(00)80439-0
- Jun, H., Bramian, A., Soma, S., Saito, T., Saido, T. C., and Igarashi, K. M. (2020). Disrupted place cell remapping and impaired grid cells in a knockin model of Alzheimer's disease. *Neuron* 107, 1095–1112.e6. doi: 10.1016/j.neuron.2020.06.023
- Kakiya, N., Saito, T., Nilsson, P., Matsuba, Y., Tsubuki, S., Takei, N., et al. (2012). Cell surface expression of the major amyloid-beta peptide (A beta)-degrading enzyme, neprilysin, depends on phosphorylation by mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) and dephosphorylation by protein phosphatase 1a. *J. Biol. Chem.* 287, 29362–29372. doi: 10.1074/jbc.M112.340372
- Karran, E., and De Strooper, B. (2016). The amyloid cascade hypothesis: are we poised for success or failure? *J. Neurochem.* 139(Suppl. 2), 237–252. doi: 10.1111/jnc.13632
- Kellar, D., and Craft, S. (2020). Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. *Lancet Neurol.* 19, 758–766. doi: 10.1016/s1474-4422(20)30231-3
- Kimura, T., Yamashita, S., Fukuda, T., Park, J. M., Murayama, M., Mizoroki, T., et al. (2007). Hyperphosphorylated tau in parahippocampal cortex impairs place learning in aged mice expressing wild-type human tau. *EMBO J.* 26, 5143–5152. doi: 10.1038/sj.emboj.7601917
- Kitazume, S., Tachida, Y., Kato, M., Yamaguchi, Y., Honda, T., Hashimoto, Y., et al. (2010). Brain endothelial cells produce amyloid [beta] from amyloid precursor protein 770 and preferentially secrete the O-glycosylated form. *J. Biol. Chem.* 285, 40097–40103. doi: 10.1074/jbc.M110.144626
- Kitazume, S., Yoshihisa, A., Yamaki, T., Oikawa, M., Tachida, Y., Ogawa, K., et al. (2012). Soluble amyloid precursor protein 770 is released from inflamed endothelial cells and activated platelets: a novel biomarker for acute coronary syndrome. *J. Biol. Chem.* 287, 40817–40825. doi: 10.1074/jbc.M112.398578
- Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., and Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 533, 420–424. doi: 10.1038/nature17946
- Kumar-Singh, S., De Jonghe, C., Cruts, M., Klei, R., Wang, R., Mercken, M., et al. (2000). Nonfibrillar diffuse amyloid deposition due to a gamma(42)-secretase site mutation points to an essential role for N-truncated a beta(42) in Alzheimer's disease. *Hum. Mol. Genet.* 9, 2589–2598. doi: 10.1093/hmg/9.18.2589
- Kwart, D., Gregg, A., Scheckel, C., Murphy, E. A., Paquet, D., Duffield, M., et al. (2019). A large panel of isogenic APP and PSEN1 mutant human iPSC neurons reveals shared endosomal abnormalities mediated by APP β -CTFs, Not A β . *Neuron* 104, 256–270.e5. doi: 10.1016/j.neuron.2019.07.010
- Kwok, J. B., Taddei, K., Hallupp, M., Fisher, C., Brooks, W. S., Broe, G. A., et al. (1997). Two novel (M233T and R278T) presenilin-1 mutations in early-onset Alzheimer's disease pedigrees and preliminary evidence for association of presenilin-1 mutations with a novel phenotype. *Neuroreport* 8, 1537–1542. doi: 10.1097/00001756-199704140-00043
- Le Guennec, K., Veugelen, S., Quenez, O., Szaruga, M., Rousseau, S., Nicolas, G., et al. (2017). Deletion of exons 9 and 10 of the presenilin 1 gene in a patient with early-onset Alzheimer disease generates longer amyloid seeds. *Neurobiol. Dis.* 104, 97–103. doi: 10.1016/j.nbd.2017.04.020

- Leissring, M. A., Farris, W., Chang, A. Y., Walsh, D. M., Wu, X., Sun, X., et al. (2003). Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron* 40, 1087–1093. doi: 10.1016/s0896-6273(03)00787-6
- Levy, E., Carman, M. D., Fernandez-Madrid, I. J., Power, M. D., Lieberburg, I., van Duinen, S. G., et al. (1990). Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* 248, 1124–1126. doi: 10.1126/science.2111584
- Li, H., Guo, Q., Inoue, T., Polito, V. A., Tabuchi, K., Hammer, R. E., et al. (2014). Vascular and parenchymal amyloid pathology in an Alzheimer disease knock-in mouse model: interplay with cerebral blood flow. *Mol. Neurodegener.* 9:28. doi: 10.1186/1750-1326-9-28
- Li, L. L., Ginet, V., Liu, X., Vergun, O., Tuittila, M., Mathieu, M., et al. (2013). The nNOS-p38MAPK pathway is mediated by NOS1AP during neuronal death. *J. Neurosci.* 33, 8185–8201. doi: 10.1523/JNEUROSCI.4578-12.2013
- Li, X. G., Hong, X. Y., Wang, Y. L., Zhang, S. J., Zhang, J. F., Li, X. C., et al. (2019). Tau accumulation triggers STAT1-dependent memory deficits by suppressing NMDA receptor expression. *EMBO Rep.* 20:e47202. doi: 10.15252/embr.201847202
- Lichtenthaler, S. F., Wang, R., Grimm, H., Uljon, S. N., Masters, C. L., and Beyreuther, K. (1999). Mechanism of the cleavage specificity of Alzheimer's disease gamma-secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein. *Proc. Natl. Acad. Sci. U S A.* 96, 3053–3058. doi: 10.1073/pnas.96.6.3053
- Lu, T., Pan, Y., Kao, S. Y., Li, C., Kohane, I., Chan, J., et al. (2004). Gene regulation and DNA damage in the ageing human brain. *Nature* 429, 883–891. doi: 10.1038/nature02661
- Maehzono, S. E. B., Kanuka, M., Tatsuzawa, C., Morita, M., Kawano, T., Kashiwagi, M., et al. (2020). Progressive changes in sleep and its relations to Amyloid- β distribution and learning in single app knock-in mice. *eNeuro* 7:ENEURO.0093-20.2020. doi: 10.1523/ENEURO.0093-20.2020
- Mansfield, K. (2003). Marmoset models commonly used in biomedical research. *Comp. Med.* 53, 383–392.
- Maruyama, M., Higuchi, M., Takaki, Y., Matsuba, Y., Tanji, H., Nemoto, M., et al. (2005). Cerebrospinal fluid neprilysin is reduced in prodromal Alzheimer's disease. *Ann. Neurol.* 57, 832–842. doi: 10.1002/ana.20494
- Masuda, A., Kobayashi, Y., Kogo, N., Saito, T., Saido, T. C., and Itohara, S. (2016). Cognitive deficits in single app knock-in mouse models. *Neurobiol. Learn. Mem.* 135, 73–82. doi: 10.1016/j.nlm.2016.07.001
- Miura, S., Yoshihisa, A., Misaka, T., Yamaki, T., Kojima, T., Toyokawa, M., et al. (2020). Amyloid precursor protein 770 is specifically expressed and released from platelets. *J. Biol. Chem.* 295, 13194–13201. doi: 10.1074/jbc.RA120.012904
- Mizuno-Iijima, S., Nakashiba, T., Ayabe, S., Nakata, H., Ike, F., Hiraiwa, N., et al. (2021). Mouse resources at the RIKEN bioresource research center and the national bioresource project core facility in Japan. *Mamm. Genome* Online ahead of print. doi: 10.1007/s00335-021-09916-x
- Møller, L. N., Stidsen, C. E., Hartmann, B., and Holst, J. J. (2003). Somatostatin receptors. *Biochim. Biophys. Acta* 1616, 1–84. doi: 10.1016/s0005-2736(03)00235-9
- Morishima-Kawashima, M., Oshima, N., Ogata, H., Yamaguchi, H., Yoshimura, M., Sugihara, S., et al. (2000). Effect of apolipoprotein E allele epsilon4 on the initial phase of amyloid beta-protein accumulation in the human brain. *Am. J. Pathol.* 157, 2093–2099. doi: 10.1016/s0002-9440(10)64847-x
- Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., et al. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nat. Genet.* 1, 345–347. doi: 10.1038/ng0892-345
- Nagata, K., Takahashi, M., Matsuba, Y., Okuyama-Uchimura, F., Sato, K., Hashimoto, S., et al. (2018). Generation of app knock-in mice reveals deletion mutations protective against Alzheimer's disease-like pathology. *Nat. Commun.* 9:1800. doi: 10.1038/s41467-018-04238-0
- Nakazono, T., Lam, T. N., Patel, A. Y., Kitazawa, M., Saito, T., Saido, T. C., et al. (2017). Impaired *in vivo* gamma oscillations in the medial entorhinal cortex of knock-in Alzheimer model. *Front. Syst. Neurosci.* 11:48. doi: 10.3389/fnsys.2017.00048
- Nalivaeva, N. N., Belyaev, N. D., Kerridge, C., and Turner, A. J. (2014). Amyloid-clearing proteins and their epigenetic regulation as a therapeutic target in Alzheimer's disease. *Front. Aging Neurosci.* 6:235. doi: 10.3389/fnagi.2014.00235
- Nilsberth, C., Westlind-Danielsson, A., Eckman, C. B., Condron, M. M., Axelman, K., Forsell, C., et al. (2001). The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nat. Neurosci.* 4, 887–893. doi: 10.1038/nn0901-887
- Nilsson, P., Sörgjerd, K., Kakiya, N., Sasaguri, H., Watanura, N., Shimozaawa, M., et al. (2020). Somatostatin receptor subtypes 1 and 4 redundantly regulate neprilysin, the major amyloid-beta degrading enzyme in brain. *bioRxiv [preprint]* doi: 10.1101/2020.05.09.085795
- Nortley, R., Korte, N., Izquierdo, P., Hirunpattarasilp, C., Mishra, A., Jaunmuktane, Z., et al. (2019). Amyloid β oligomers constrict human capillaries in Alzheimer's disease via signaling to pericytes. *Science* 365:eaav9518. doi: 10.1126/science.aav9518
- Okano, H. (2021). Current status of and perspectives on the application of marmosets in neurobiology. *Annu. Rev. Neurosci.* 44, 27–48. doi: 10.1146/annurev-neuro-030520-101844
- Park, J. E., and Sasaki, E. (2021). Assisted reproductive techniques and genetic manipulation in the common marmoset. *Ilar J.* Online ahead of print. doi: 10.1093/ilar/ilab002
- Peng, W., Achariyar, T. M., Li, B., Liao, Y., Mestre, H., Hitomi, E., et al. (2016). Suppression of glymphatic fluid transport in a mouse model of Alzheimer's disease. *Neurobiol. Dis.* 93, 215–225. doi: 10.1016/j.nbd.2016.05.015
- Prihar, G., Verkoniem, A., Perez-Tur, J., Crook, R., Lincoln, S., Houlden, H., et al. (1999). Alzheimer disease PS-1 exon 9 deletion defined. *Nat. Med.* 5:1090. doi: 10.1038/13383
- Pyun, J. M., Kang, M. J., Yun, Y., Park, Y. H., and Kim, S. (2019). APOE ϵ 4 and REM sleep behavior disorder as risk factors for sundown syndrome in Alzheimer's disease. *J. Alzheimers. Dis.* 69, 521–528. doi: 10.3233/jad-190032
- Qiu, W. Q., Walsh, D. M., Ye, Z., Vekrellis, K., Zhang, J., Podlisny, M. B., et al. (1998). Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J. Biol. Chem.* 273, 32730–32738. doi: 10.1074/jbc.273.49.32730
- Richier, L., Williton, K., Clattenburg, L., Colwill, K., O'Brien, M., Tsang, C., et al. (2010). NOS1AP associates with scribble and regulates dendritic spine development. *J. Neurosci.* 30, 4796–4805. doi: 10.1523/JNEUROSCI.3726-09.2010
- Roberson, E. D., Searce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., et al. (2007). Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* 316, 750–754. doi: 10.1126/science.1141736
- Rodriguez-Callejas, J. D., Fuchs, E., and Perez-Cruz, C. (2016). Evidence of tau hyperphosphorylation and dystrophic microglia in the common marmoset. *Front. Aging Neurosci.* 8:315. doi: 10.3389/fnagi.2016.00315
- Rosario, D., Boren, J., Uhlen, M., Proctor, G., Aarsland, D., Mardinoglu, A., et al. (2020). Systems biology approaches to understand the host-microbiome interactions in neurodegenerative diseases. *Front. Neurosci.* 14:716. doi: 10.3389/fnins.2020.00716
- Saito, T., Iwata, N., Tsubuki, S., Takaki, Y., Takano, J., Huang, S. M., et al. (2005). Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation. *Nat. Med.* 11, 434–439. doi: 10.1038/nm1206
- Saito, T., Matsuba, Y., Mihira, N., Takano, J., Nilsson, P., Itohara, S., et al. (2014). Single App knock-in mouse models of Alzheimer's disease. *Nat. Neurosci.* 17, 661–663. doi: 10.1038/nn.3697
- Saito, T., Matsuba, Y., Yamazaki, N., Hashimoto, S., and Saido, T. C. (2016). Calpain activation in Alzheimer's model mice is an artifact of APP and presenilin overexpression. *J. Neurosci.* 36, 9933–9936. doi: 10.1523/jneurosci.1907-16.2016
- Saito, T., Mihira, N., Matsuba, Y., Sasaguri, H., Hashimoto, S., Narasimhan, S., et al. (2019). Humanization of the entire murine Mapt gene provides a murine model of pathological human tau propagation. *J. Biol. Chem.* 294, 12754–12765. doi: 10.1074/jbc.RA119.009487
- Salobrar-García, E., López-Cuenca, I., Sánchez-Puebla, L., de Hoz, R., Fernández-Albarral, J. A., Ramírez, A. I., et al. (2020). Retinal thickness changes over time in a murine AD model APP (NL-F/NL-F). *Front. Aging Neurosci.* 12:625642. doi: 10.3389/fnagi.2020.625642
- Saria, A., Hauser, K. F., Traurig, H. H., Turbek, C. S., Hersh, L., and Gerard, C. (1997). Opioid-related changes in nociceptive threshold and in tissue levels of enkephalins after target disruption of the gene for neutral endopeptidase

- (EC 3.4.24.11) in mice. *Neurosci. Lett.* 234, 27–30. doi: 10.1016/s0304-3940(97)00660-5
- Sasaguri, H., Nagata, K., Sekiguchi, M., Fujioka, R., Matsuba, Y., Hashimoto, S., et al. (2018). Introduction of pathogenic mutations into the mouse *Psen1* gene by base editor and Target-AID. *Nat. Commun.* 9:2892. doi: 10.1038/s41467-018-05262-w
- Sasaguri, H., Nilsson, P., Hashimoto, S., Nagata, K., Saito, T., De Strooper, B., et al. (2017). APP mouse models for Alzheimer's disease preclinical studies. *EMBO J.* 36, 2473–2487. doi: 10.15252/embj.201797397
- Sasaki, E., Suemizu, H., Shimada, A., Hanazawa, K., Oiwa, R., Kamioka, M., et al. (2009). Generation of transgenic non-human primates with germline transmission. *Nature* 459, 523–527. doi: 10.1038/nature08090
- Sato, K., Oiwa, R., Kumita, W., Henry, R., Sakuma, T., Ito, R., et al. (2016). Generation of a nonhuman primate model of severe combined immunodeficiency using highly efficient genome editing. *Cell Stem Cell* 19, 127–138. doi: 10.1016/j.stem.2016.06.003
- Sato, K., Sasaguri, H., Kumita, W., Inoue, T., Kurotaki, Y., Nagata, K., et al. (2020). A non-human primate model of familial Alzheimer's disease. *bioRxiv [preprint]* doi: 10.1101/2020.08.24.264259
- Sato, K., Watamura, N., Fujioka, R., Mihira, N., Sekiguchi, M., Nagata, K., et al. (2021). A 3(rd) generation mouse model of Alzheimer's disease shows early and increased cored plaque pathology composed of wild-type human amyloid β peptide. *J. Biol. Chem.* 297:101004. doi: 10.1016/j.jbc.2021.10.1004
- Searce-Levie, K., Sanchez, P. E., and Lewcock, J. W. (2020). Leveraging preclinical models for the development of Alzheimer disease therapeutics. *Nat. Rev. Drug Discov.* 19, 447–462. doi: 10.1038/s41573-020-0065-9
- Selkoe, D. J., and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* 8, 595–608. doi: 10.15252/emmm.201606210
- Shirohori, K., Hori, Y., Yoshizaki, R., Higuchi, E., Colonna, M., Saito, T., et al. (2019). Aminophospholipids are signal-transducing TREM2 ligands on apoptotic cells. *Sci. Rep.* 9:7508. doi: 10.1038/s41598-019-43535-6
- Smith, M. J., Kwok, J. B., McLean, C. A., Kril, J. J., Broe, G. A., Nicholson, G. A., et al. (2001). Variable phenotype of Alzheimer's disease with spastic paraparesis. *Ann. Neurol.* 49, 125–129.
- Sobue, A., Komine, O., Hara, Y., Endo, F., Mizoguchi, H., Watanabe, S., et al. (2021). Microglial gene signature reveals loss of homeostatic microglia associated with neurodegeneration of Alzheimer's disease. *Acta Neuropathol. Commun.* 9:1. doi: 10.1186/s40478-020-01099-x
- Steiner, H., Romig, H., Grim, M. G., Philipp, U., Pesold, B., Citron, M., et al. (1999). The biological and pathological function of the presenilin-1 Deltaexon 9 mutation is independent of its defect to undergo proteolytic processing. *J. Biol. Chem.* 274, 7615–7618. doi: 10.1074/jbc.274.12.7615
- Susaki, E. A., Shimizu, C., Kuno, A., Tainaka, K., Li, X., Nishi, K., et al. (2020). Versatile whole-organ/body staining and imaging based on electrolyte-gel properties of biological tissues. *Nat. Commun.* 11:1982. doi: 10.1038/s41467-020-15906-5
- Sutoko, S., Masuda, A., Kandori, A., Sasaguri, H., Saito, T., Saido, T. C., et al. (2021). Early identification of Alzheimer's disease in mouse models: application of deep neural network algorithm to cognitive behavioral parameters. *iScience* 24:102198. doi: 10.1016/j.isci.2021.102198
- t Hart, B. A., and Massacesi, L. (2009). Clinical, pathological, and immunologic aspects of the multiple sclerosis model in common marmosets (*Callithrix jacchus*). *J. Neuropathol. Exp. Neurol.* 68, 341–355. doi: 10.1097/NEN.0b013e31819fd24
- Tachida, Y., Miura, S., Imamaki, R., Ogasawara, N., Takuwa, H., Sahara, N., et al. (2020). Endothelial expression of human APP leads to cerebral amyloid angiopathy in mice. *bioRxiv [preprint]* doi: 10.1101/2020.07.22.215418
- Takamura, R., Mizuta, K., Sekine, Y., Islam, T., Saito, T., Sato, M., et al. (2021). Modality-Specific impairment of hippocampal CA1 neurons of Alzheimer's disease model mice. *J. Neurosci.* 41, 5315–5329. doi: 10.1523/jneurosci.0208-21.2021
- Tardif, S. D., Mansfield, K. G., Ratnam, R., Ross, C. N., and Ziegler, T. E. (2011). The marmoset as a model of aging and age-related diseases. *ILAR J.* 52, 54–65. doi: 10.1093/ilar.52.1.54
- Tardif, S. D., Smucny, D. A., Abbott, D. H., Mansfield, K., Schultz-Darken, N., and Yamamoto, M. E. (2003). Reproduction in captive common marmosets (*Callithrix jacchus*). *Comp. Med.* 53, 364–368.
- Tsubuki, S., Takaki, Y., and Saido, T. C. (2003). Dutch, Flemish, Italian, and Arctic mutations of APP and resistance of Abeta to physiologically relevant proteolytic degradation. *Lancet* 361, 1957–1958. doi: 10.1016/s0140-6736(03)13555-6
- Turner, A. J., and Nalivaeva, N. N. (2006). Proteinase dysbalance in pathology: the neprilysin (NEP) and angiotensin-converting enzyme (ACE) families. *Cell Mol. Biol. (Noisy-le-grand)* 52, 40–48.
- Turner, A. J., Brown, C. D., Carson, J. A., and Barnes, K. (2000). The neprilysin family in health and disease. *Adv. Exp. Med. Biol.* 477, 229–240. doi: 10.1007/0-306-46826-3_25
- Turner, A. J., Isaac, R. E., and Coates, D. (2001). The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *Bioessays* 23, 261–269. doi: 10.1002/1521-1878(200103)23:3<261::AID-BIES1036>3.0.CO;2-K
- Turner, A. J., Murphy, L. J., Medeiros, M. S., and Barnes, K. (1996). Endopeptidase-24.11 (neprilysin) and relatives: twenty years on. *Adv. Exp. Med. Biol.* 389, 141–148. doi: 10.1007/978-1-4613-0335-0_17
- Urano, A., Matsumaru, D., Ryoike, R., Saito, R., Kadoguchi, S., Saigusa, D., et al. (2020). Nrf2 suppresses oxidative stress and inflammation in app knock-in Alzheimer's disease model mice. *Mol. Cell Biol.* 40:e00467-19. doi: 10.1128/mcb.00467-19
- Van Broeckhoven, C., Haan, J., Bakker, E., Hardy, J. A., Vanhul, W., Wehnert, A., et al. (1990). Amyloid-Beta protein-precursor gene and hereditary cerebral hemorrhage with amyloidosis (DUTCH). *Science* 248, 1120–1122. doi: 10.1126/science.1971458
- van de Nes, J. A., Sandmann-Keil, D., and Braak, H. (2002). Interstitial cells subjacent to the entorhinal region expressing somatostatin-28 immunoreactivity are susceptible to development of Alzheimer's disease-related cytoskeletal changes. *Acta Neuropathol.* 104, 351–356. doi: 10.1007/s00401-002-0551-7
- Vepsäläinen, S., Helisalmi, S., Koivisto, A. M., Tapaninen, T., Hiltunen, M., and Soininen, H. (2007). Somatostatin genetic variants modify the risk for Alzheimer's disease among Finnish patients. *J. Neurol.* 254, 1504–1508. doi: 10.1007/s00415-007-0539-2
- Watamura, N., Kakiya, N., Nilsson, P., Tsubuki, S., Kamano, N., Takahashi, M., et al. (2021a). Somatostatin-evoked A β catabolism in the brain: mechanistic involvement of α -endosulfine-KATP channel pathway. *Mol. Psychiatry* Online ahead of print. doi: 10.1038/s41380-021-01368-8
- Watamura, N., Sato, K., Shiihashi, G., Iwasaki, A., Kamano, N., Takahashi, M., et al. (2021b). An isogenic panel of single app knock-in mouse models of Alzheimer's disease confers differential profiles of β -secretase inhibition and endosomal abnormalities. *bioRxiv [preprint]* doi: 10.1101/2021.08.22.457278
- Xia, D., Lianoglou, S., Sandmann, T., Calvert, M., Suh, J. H., Thomsen, E., et al. (2021). Fibrillar A β causes profound microglial metabolic perturbations in a novel APP knock-in mouse model. *bioRxiv [preprint]* doi: 10.1101/2021.01.19.426731
- Yasojima, K., Akiyama, H., McGeer, E. G., and McGeer, P. L. (2001). Reduced neprilysin in high plaque areas of Alzheimer brain: a possible relationship to deficient degradation of beta-amyloid peptide. *Neurosci. Lett.* 297, 97–100. doi: 10.1016/s0304-3940(00)01675-x
- Zhang, H. X., Zhang, Y., and Yin, H. (2019). Genome editing with mRNA encoding ZFN, TALEN, and Cas9. *Mol. Ther.* 27, 735–746.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Sasaguri, Hashimoto, Watamura, Sato, Takamura, Nagata, Tsubuki, Ohshima, Yoshiki, Sato, Kumita, Sasaki, Kitazume, Nilsson, Winblad, Saito, Iwata and Saido. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.