Graduate School of Advanced Science and Engineering
Waseda University

博 士 論 文 概 要
Doctoral Thesis Synopsis

論 文 題 目
Thesis Theme

Pathological and Functional Analysis of Combined Mutations
Associated with Alzheimer's Disease in Mice

アルツハイマー病の複合変異が生体にもたらす
病理学的および機能的影響の解析

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Alzheimer's disease (AD), the most prevalent cause of dementia, has been intensively investigated worldwide for over 100 years since it was first reported by Dr. Aois Alzheimer. Patients suffered from AD irreversibly lose cognitive ability as the disease progresses, which means that the disease catastrophically deprives patients of human dignity and torments patients and their families. There are currently, however, no efficacious disease-modifying treatments available for AD, although aducanumab, an anti- amyloid β peptide (Aβ) human monoclonal antibody, was approved for use by the U.S. Food and Drug Administration in June 2021 following positive Phase 4 trial outcomes.

The major pathological hallmark of AD is deposition of plaques primarily composed of Aβ in the brain. Dozens of studies targeting Aβ have been carried out worldwide, because excess Aβ initiates to accumulate more than 20 years before symptoms appear. Genetic studies have revealed that pathogenic mutations alter Aβ profile produced in the brain, leading to early onset of AD. More than 350 genetic variants have been identified in the *presenilin 1* (*PSEN1*) and in the *amyloid precursor protein* (*APP*) genes. *PSEN1* encodes a catalytic subunit of the secretase responsible for Aβ production.

To date, significant research advances have been achieved thanks to mouse models that recapitulate aspects of the AD pathophysiology seen in humans. Most AD models overexpress mutant *APP* or *APP*/*PS1* complementary DNAs. Such mouse models, however, often suffer from experimental limitations caused by overproduction of APP fragments such as C-terminal fragment of APP generated by β-site APP cleaving enzyme 1 (BACE1) (CTF-β) and APP intracellular domain, both of which do not appear to accumulate in AD brains. To overcome these drawbacks, *App* knock-in mice such as App^{NL-G-F} and App^{NL-F} mice were previously developed in 2014. These mice harbor the Swedish (KM670/671NL) and Beyreuther/Iberian (I716F) mutations – with or without the Arctic (E693G) mutation – that do not depend on overexpression paradigm. These models showed the development of Aβ pathology, neuroinflammation and cognitive deficits with aging. The App^{NL-G-F} line has been more frequently used than the App^{NL-F} line because the former develops Aβ pathology much faster than the latter and can be conveniently used to analyze downstream events.

However, the *App* knock-in models are still burdened by at least two problems as follows: [1] The App^{NL-G-F} line produce the mutant Arctic Aβ, not wild-type human

Aβ deposited in AD patients, because the Arctic mutation exists within Aβ sequence, which makes the model unsuitable for investigating Aβ metabolism and clearance because the Arctic mutation renders Aβ resistant to proteolytic degradation and prone to aggregation. In contrast, the App^{NL-F} line does accumulate wild-type human Aβ, but it takes as long as approximately 18 months for the pathology to become prominent. [2] These models are inappropriate for use in preclinical studies of BACE1 inhibitors because the Swedish mutation, located adjacent to the cleavage site of APP by BACE1, results in a drastic increase in CTF-β levels and influences the mode of BACE1 inhibitor action *in vivo*. In addition, although recent cell-based studies have reported that CTF-β, not Aβ, contributes to aberrant events in the endosomal trafficking system that may appear as a common cytopathology, it is not well understood if CTF-β affects early endosomal dysfunction *in vivo*.

The aim of the present study was thus to generate two additional lines of *App* knock-in mouse models for exploring the functional consequences of novel combinations of pathogenic mutations *in vivo*: [1] A new *App* knock-in model that exhibits earlier accumulation of wild-type human Aβ than the App^{NL-F} model without depending on the Arctic mutation. [2] A new *App* knock-in model devoid of the Swedish mutation that is useful for preclinical studies of BACE1 inhibitors and for examination of early endosome profile *in vivo*.

This thesis consists of five chapter. The contents are summarized as follows.

Chapter 1 including six sections describes the introduction of this thesis. Each section explains AD (1.1), APP (1.2), PSEN1 (1.3), genetic variants (1.4), mouse models (1.5) genome editing technology (1.6) and endosomal trafficking (1.7), respectively.

Chapter 2 gives the details of experimental methods that were utilized in the present study.

Chapter 3 is a result part which are divided into two sections.

In the first section (3.1), I presented the effect of the novel combination of pathogenic mutations in the *App* and *Psen1* genes on amyloid pathology *in vivo*. I devised the strategy to utilize the heterozygous $Psen1^{P117L/WT}$ mutant line ($Psen1^{P117L}$) that exhibited the largest increase in $Aβ_{42}/Aβ_{40}$ ratio in the brain among several *Psen1* mutants. I attempted to crossbreed App^{NL-F} mice with $Psen1^{P117L}$ mice despite it being unclear whether their pathogenic effects, both of which act on the γ-cleavage of CTF-β, are additive or not *in vivo*. I demonstrate here that the combined $Psen1^{P117L}$ mutation markedly enhances the pathological phenotypes of App^{NL-F} mice additively

or synergistically.

In the second section (3.2), I showed the functional consequences of combinedly pathogenic mutations derived from the *App* knock-in mice without the presence of Swedish mutation (App^{G-F} mice) and some additional mutants. I used a CRISPR/Cas9 system to develop $App^{G-F/G-F}$ knock-in (App^{G-F}) mice harboring the Arctic and Beyreuther/Iberian mutations but devoid of the Swedish mutations. Similar to the App^{NL-F} and App^{NL-G-F} lines, a combination of the Arctic and Iberian mutations induces an age-dependent amyloid pathology, neuroinflammation and synaptic alteration in mouse brains. App^{G-F} mice is the first AD mouse model ever described that recapitulates amyloid pathology in the brain without the use of Swedish mutations and without relying on the overexpression paradigm. Acute administration of verubecestat, a potent selective BACE1 inhibitor, reduced Aβ levels in App^{G-F} mice, but not in App^{NL-G-F} mice. I also found that early endosomal enlargement was present in the brains of App^{G-F} mice even though the CTF-β quantity was quantitatively comparable to that of WT mice. These findings demonstrate that BACE1 activity can be appropriately evaluated in App^{G-F} mice without the interference of the Swedish mutations and that endosome enlargement does not correlate with CTF-β levels *in vivo*.

Chapter 4 discusses the pros and cons of the new *App* knock-in mice that I generated. This chapter is also divided into two sections, corresponding to the section number indicated in Chapter 3. (4.1) I anticipate that the double mutant mice will become highly relevant tools for examining the mechanisms upstream of Aβ deposition, for preclinical screening of disease-modifying therapy candidates, which for instance promote Aβ degradation or disaggregation, and for preclinical immunotherapy studies without any concern regarding the artificial effect of the Arctic mutation. In addition (4.2), the findings demonstrate that BACE1 activity can be appropriately evaluated in App^{G-F} mice without the interference of the Swedish mutations and that endosome enlargement does not correlate with CTF-β levels *in vivo*. Experimental comparisons between different *App* knock-in mouse lines will potentially provide new insights into our understanding of the etiology of AD.

Chapter 5 summarizes the essence of the whole story and the roles of animal models in AD drug development. Regardless of depending on the overexpression or knock-in paradigm, it should be noted that users choose which line most suited to the purpose of their studies especially at preclinical study stages for the development of disease-modifying therapies.

List of research achievements for application of Doctor of Science, Waseda University

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種類別 (By Type)	題名、　発表・発行掲載誌名、　　発表・発行年月、　　連名者（申請者含む） (theme, journal name, date & year of publication, name of authors inc. yourself)
Academic papers ○	**Kaori Sato***, Naoto Watamura*, Ryo Fujioka, Naomi Mihira, Misaki Sekiguchi, Kenichi Nagata, Toshio Ohshima, Takashi Saito, Takaomi C. Saido and Hiroki Sasaguri. A 3rd 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(3):101004 (2021)　*These authors contributed equally to this work.
Academic papers	Kenichi Nagata, Mika Takahashi, Yukio Matsuba, Fumi Okuyama-Uchimura, **Kaori Sato**, Shoko Hashimoto, Takashi Saito and Takaomi C. Saido. Generation of App knock-in mice reveals deletion mutations protective against Alzheimer's disease-like pathology. *Nature Communications* , 9(1):1800 (2018)
Academic papers	Hiroki Sasaguri, Kenichi Nagata, Misaki Sekiguchi, Ryo Fujioka, Yukio Matsuba, Shoko Hashimoto, **Kaori Sato**, Deepika Kurup, Takanori Yokota and Takaomi C. Saido. Introduction of pathogenic mutations into the mouse Psen1 gene by Base Editor and Target-AID. *Nature Communications* , 9(1):2892 (2018)
Lectures	**Kaori Sato**. Recent advancement in modeling Alzheimer's disease. The CBS Young Investigators' Seminar, Saitama, Japan, 2021.6 (oral)
Lectures	**Kaori Sato**, Kenichi Nagata, Takaomi Saido, Hiroki Sasaguri. The combined effects of pathogenic mutations in the App and Psen1 genes in vivo. The 39th annual meeting of Japan society for dementia research. Aichi, Japan (changed to hybrid conference due to COVID-19 pandemic), 2020.11 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Takaomi Saido, Hiroki Sasaguri. The effects of pathogenic mutations in the App and Psen1 genes on in vivo γ-secretase activity are additive. Alzheimer's association international conference 2020, Amsterdam, Netherland (unfortunately changed to virtual conference due to COVID-19 pandemic), 2020.7 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. 内在性プレセニリン1エクソン9欠損マウスの作製と機能解析. The 38th annual meeting of Japan society for dementia research. Tokyo, Japan, 2019.11 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. Introduction of deletion mutation associated with Alzheimer's disease into mouse genome. Neuroscience 2019, Chicago, America, 2019.10 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. Introduction of deletion mutation associated with Alzheimer's disease into mouse genome. The international fellows poster session at neuroscience 2019. Chicago, America, 2019.10 (poster)

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Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. CRISPR/Cas-mediated generation of Presenilin1 mutant mouse models with Alzheimer's disease associated mutations. The 71st annual scientific meeting of the American association for clinical chemistry. Anaheim, America, 2019.8 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. CRISPR/Cas-mediated generation of Presenilin1 mutant mouse models with Alzheimer's disease associated mutations. The 2019 AACC student poster conference. Anaheim, America, 2019.8 (poster)
Lectures	**Kaori Sato**, Hiroki Sasaguri, Kenichi Nagata, Takaomi Saido. Introduction of deletion mutations associated with Alzheimer's disease into mouse genome. 2019年度革新脳 後期キックオフシンポジウム. Kanagawa, Japan, 2019.7 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. CRISPR/Cas-mediated generation of Presenilin1 mutant mouse models with Alzheimer's disease associated mutations. The 2019 Joint Annual Meeting of the Japan Neuroscience Society and the Japanese Society for Neurochemistry. Niigata, Japan, 2019.7 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. Targeted genomic deletion associated with Alzheimer's disease in mouse. The 4th annual meeting of Japan society for genome editing. Tokyo, Japan, 2019.6 (poster)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Oshima, Takaomi Saido. ゲノム編集技術による Psen1変異ノックインモデルの作製. The 37th annual meeting of Japan society for dementia research. Hokkaido, Japan. 2018.10 (poster)
Lectures	**Kaori Sato**. ゲノム編集技術による Psen1変異ノックインモデルの作製. 第8回認知症研究を知る若手研究者の集まり2018. Shiga, Japan. 2018.7 (oral)
Lectures	**Kaori Sato**, Kenichi Nagata, Hiroki Sasaguri, Toshio Ohshima, Takaomi Saido. A mutant cell line of Alzheimer's disease-associated Presnilin-1 mutation using CRISPR-Cas system. The 4th annual meeting of Japan society for genome editing. Hiroshima, Japan, 2018.6 (poster)