ACS Chemical Neuroscience

Viewpoint

pubs.acs.org/chemneuro

New Mouse Model of Alzheimer's

Per Nilsson,*[,†] Takashi Saito,[†,‡] and Takaomi C Saido*[,†]

[†]Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

[‡]Japan Science and Technology Agency, Kawaguchi-shi, Saitama 332-0012, Japan

ABSTRACT: Amyloid β-peptide (Aβ) accumulation is a key characteristic of Alzheimer's disease (AD); therefore, mouse models of AD exhibiting Aβ pathology are valuable tools for unraveling disease mechanisms. However, the overexpression of Aβ precursor protein (APP) used in previous mouse models may cause Aβ-independent artifacts that influence data interpretation. To circumvent these problems, we used an APP knock-in (KI) strategy to introduce mutations to the mouse APP gene to develop a new generation of AD mouse models. These new models, termed APP^{NL-F} and APP^{NL-G-F}, have endogenous APP levels and develop robust Aβ amyloidosis, which induce synaptic degeneration and memory impairments. Thus, we suggest that these novel APP KI mice will serve as important tools to elucidate molecular mechanisms of AD.

KEYWORDS: *Alzheimer's disease, mouse models, APP knock-in, Aβ*

Even though Alzheimer's disease (AD) was first described over 100 years ago, a disease-modifying treatment has not yet been developed for this disease, which accounts for the majority of dementia in the elderly. The main risk factor for AD is aging; taking the rapidly increasing life span into account, the need for a cure is obviously high. To elucidate AD mechanisms, a number of mouse models have been developed. However, the majority of these are transgenic (Tg) mouse models which employ overexpression paradigms. This unphysiological production of proteins most likely induces artifacts that hinder the interpretation of the data obtained from these mice. Therefore, our laboratory has generated a new generation of mouse models of AD, using a knock-in (KI) approach that circumvent the drawbacks of the overexpression models.

The brains of AD patients are characterized by two pathological hallmarks: aggregates of amyloid β-peptide (Aβ), which form extracellular plaques, and intracellular accumulation of hyperphosphorylated protein tau, so-called neurofibrillary tangles (NFTs). Although the molecular link between Aβ and tau has yet to be elucidated, the amyloid cascade hypothesis postulates that increased Aβ levels precedes tau aggregation, especially in the cortical region.[1] This is supported by the fact that familial AD mutations in Aβ precursor protein (APP) or in either presenilin 1 (PS1) or PS2, the catalytic entities of γ-secretase that proteolytically process APP to Aβ, increase Aβ or the Aβ42/40 ratio leading to both Aβ plaques and NFTs. On the other hand, mutations in tau associated with frontotemporal dementia do not induce Aβ plaques: consequently, NFTs may exist in the absence of Aβ plaque. In addition, the recent finding that a mutation (A673T) in APP, which lowers Aβ levels, is protective against AD strongly associates Aβ to the development of AD.[2]

Mouse models are powerful resources for elucidating human disease mechanisms. Previous AD mouse models have generated a wealth of information that has significantly improved the knowledge of AD. However, the majority of these models are based on transgenic overexpression of APP in combinations with different familial AD-associated mutations in APP or PS1. This overexpression of APP generates elevated Aβ levels to mimic the Aβ amyloidosis of AD brains. However, due to APP's multiple roles in the cell, unphysiologically high levels of APP caused by the overexpression also induces a number of undesirable side effects (Figure 1A, Table 1).[3] Although the precise biological function for APP is yet to be clarified, it has been established that APP knockout (KO) mice exhibit lowered locomotor activity and decreased long-term potentiation (possibly due to the role of APP in cell and synaptic adhesion). APP also interacts with kinesin, via JIP-1; therefore, APP overexpression may interfere with transport machinery in the cell. Another sign of perturbed physiology caused by APP overexpression is the sudden death often observed in APP Tg mice. In addition, the insertion of the APP gene into the host DNA may alter the endogenous gene expression. Furthermore, the expression is often driven from artificial promoters, which leads to APP production in cell types that would not normally express APP, and these artificial promoters may also compete with the endogenous promoters for transcription factors. Additionally, cell-type specific splicing may be spared. Importantly, APP is proteolytically cleaved at several positions; thus, overexpression of APP automatically generates increased levels of APP fragments including sAPP, CTF-α, CTF-β, and AICD. At least some of these fragments have biological functions: for example, sAPP may have a neurotrophic effect whereas CTF-β negatively affects memory. In summary, the numerous potential artifacts associated with APP overexpression in APP Tg mice warranted the development of a new generation of AD mouse models exhibiting robust Aβ amyloidosis without APP overexpression.

A key characteristic of AD brains is the increased levels of Aβ, especially the hydrophobic and aggregation-prone Aβ42. To generate a novel AD mouse model exhibiting increased Aβ42 levels without APP overexpression, we applied a KI strategy. The Aβ sequence spans over two exons in the APP gene separated by intron 16. During the generation of the APP KI

Received: May 11, 2014
Revised: May 12, 2014
Published: May 22, 2014

dx.doi.org/10.1021/cn500105p | *ACS Chem. Neurosci.* 2014, 5, 499−502

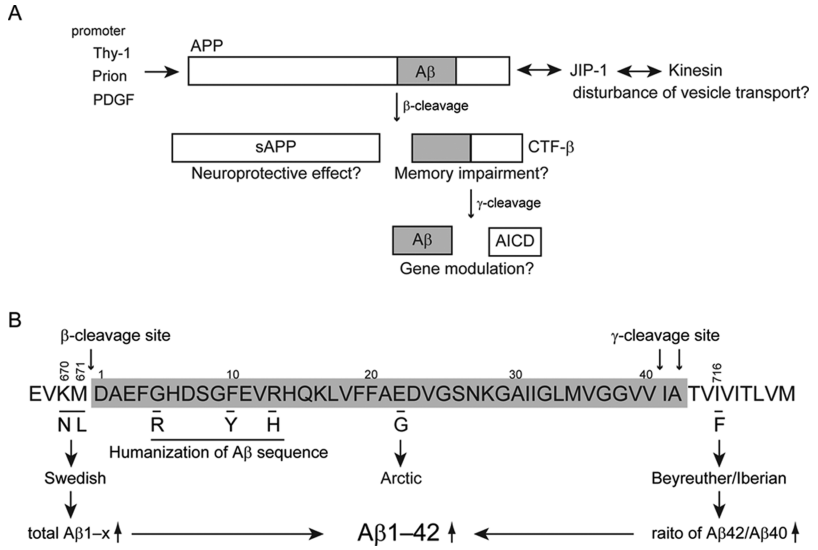

Figure 1. Concept of novel APP KI mice. (A) APP Tg mice that overexpress APP exhibit not only unphysiologically high levels of APP but also increased levels of the proteolytical fragments of APP. This may induce Aβ-independent artifacts that hinder the interpretation of data from APP Tg mice. (B) Design of APP KI mice. The Aβ sequence was humanized, and the Swedish and the Beyreuther/Iberian mutations were introduced into the APP mouse gene to generate $APP^{NL\text{-}F}$ mice. In addition, the arctic mutation was introduced to establish the $APP^{NL\text{-}G\text{-}F}$ mouse model.

Table 1. Intrinsic Problems of APP Tg Mice

(1)	APP overexpression interferes with intracellular transport because APP interacts with kinesin via JIP-1.
(2)	Due to the proteolytic processing of APP, increased APP levels leads to increased sAPP, CTF-β, CTF-α, and AICD, some of which may have biological functions.
(3)	Utilization of artificial promoters induces expression of APP in cell types normally not expressing APP. This artificial expression of APP may affect these cells in an unaccounted-for manner and also lead to the omission of the physiological cell type-specific splicing.
(4)	Artificial promoters compete with endogenous promoters, hence affecting gene expression.
(5)	Insertion of the APP transgene may destroy or alter gene expression of the host DNA.
(6)	The expression of transgene APP varies from line to line and over time.
(7)	APP Tg mouse lines are selected in a phenotype-based manner rather than by genotype.
(8)	APP Tg mice suddenly die prematurely, possibly from seizures.
(9)	The use of homozygous mice is not relevant.
(10)	Cross breeding APP Tg mice with other mutant mice may induce even more complicated artifacts.

mouse we found that this intron exhibited a regulatory function important for proper APP expression and was hence indispensable (data not shown). Along with humanizing the Aβ sequence, we introduced the Swedish mutation (KM670/671NL) and the Beyreuther/Iberian mutation (I716F) into exon 16 and exon 17, respectively, of the mouse APP gene[3] (Figure 1B). We named this mouse $APP^{NL\text{-}F}$. The Swedish mutation increases β site cleavage of APP, leading to increased APP-CTF-β levels that in turn augments both Aβ40 and Aβ42 levels, whereas the Beyreuther/Iberian mutation increases γ-cleavage at the C-terminal position 42, which specifically increases the Aβ42/Aβ40 ratio. We also created an APP KI mouse line that contains only the Swedish mutation, named APP^{NL}. Because APP^{NL} mice exhibit the same levels of CTF-β, this APP KI mouse line serves as a proper negative control for $APP^{NL\text{-}F}$ mice. Careful examination of the $APP^{NL\text{-}F}$ mice confirmed a high level of Aβ42 and a high Aβ42/40 ratio, which induced plaque formation at six months of age. Consistently, the major species in the plaques was Aβ42, similar to the Aβ pathology observed in AD brains (Figure 2A).[4] Furthermore, significant accumulation of both astrocytes and microglia was observed around the plaques (Figure 2B). In addition, synaptic alterations occurred as manifested by decreases in the presynaptic synoptophysin and postsynaptic protein PSD95. These Aβ-induced pathologies lead to impaired memory at 18 months of age in the $APP^{NL\text{-}F}$ mice (Figure 2C). We additionally created an APP KI line that also includes the Arctic mutation termed $APP^{NL\text{-}G\text{-}F}$. APP processing in this mouse was similar to $APP^{NL\text{-}F}$ mice. Remarkably, the $APP^{NL\text{-}G\text{-}F}$ mice showed very early and aggressive Aβ pathology from 2 months of age, including subcortical depositions (Figure 2D). This pathology led to earlier onset of astrocytosis and gliosis compared to $APP^{NL\text{-}F}$ mice, indicating that Aβ with the arctic mutation results in an increased inflammatory response. Notably, the pathologies induce memory impairment as early as 6 months of age (Figure 2E,F).

We have previously shown that deficiency of calpastatin (Cast), an endogenous inhibitor of calpain, leads to increased Aβ and tau pathologies and early lethality in APP Tg mice.[5] To validate whether these pathologies were indeed induced by Aβ, we crossed $APP^{NL\text{-}F}$ mice with calpastatin KO mice. To our surprise, the $APP^{NL\text{-}F} \times$ Cast $KO^{-/-}$ mice exhibited normal life span and lacked increased tau pathologies but exhibited increased Aβ amyloidosis. This is the first application of our new APP KI mouse, which shows that the early lethality of calpastatin deficiency in APP Tg mice was not caused by Aβ but was due to the overexpression of APP and/or proteolytic fragments thereof. We believe that our novel APP KI mice will provide new standard models for AD research and serve as important tools to identify potential artifacts in previously obtained data using APP Tg mice. Crossbreeding the APP KI mice with other mutant mice will unambiguously clarify mechanisms and pathways upstream and downstream of Aβ, which will contribute to the understanding of AD.

dx.doi.org/10.1021/cn500105p | ACS Chem. Neurosci. 2014, 5, 499−502

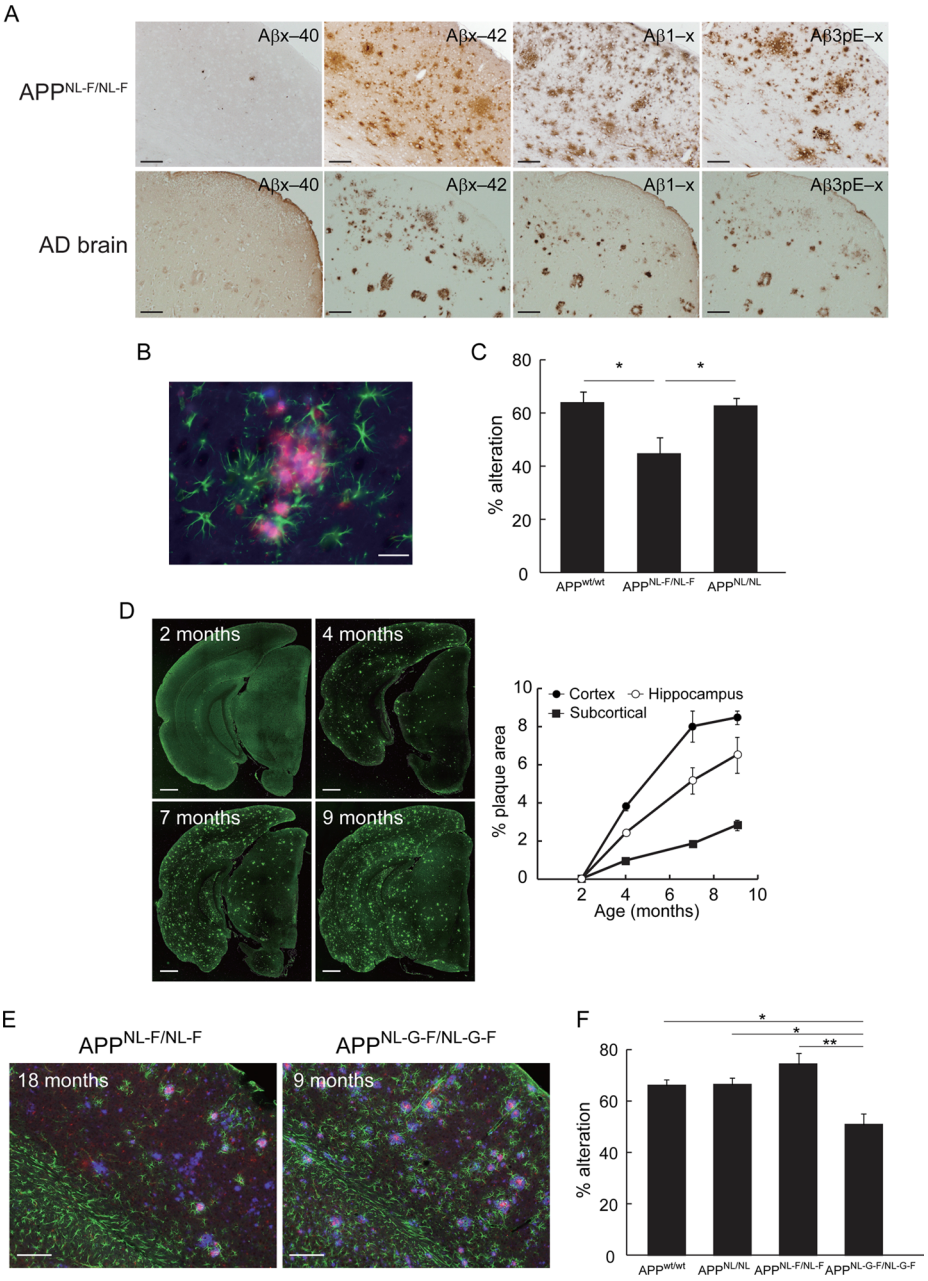

Figure 2. Aβ pathologies and neuropathologies of APP^{NL-F} and APP^{NL-G-F} mice. (A) Aβ plaque staining of APP^{NL-F} and AD brains, showing that Aβ42 is the major species in both APP^{NL-F} mice and AD brains. In addition, N-terminal processing of Aβ generates Aβ3pE-Aβ. (B) Increased astrocytosis and gliosis around the Aβ plaques in APP^{NL-F} mice. (C) Aβ amyloidosis induces memory impairment in 18 month old APP^{NL-F} mice (Y-maze test). Aβ plaque staining of APP^{NL-G-F} mice shows early and aggressive Aβ amyloidosis (D) accompanied by astrocytosis/gliosis (E). (F) Pathologies induce memory impairment in 6 month old APP^{NL-G-F} mice (Y-maze test).

AUTHOR INFORMATION

Corresponding Authors

*E-mail: per-nilsson@brain.riken.jp.
*E-mail: saido@brain.riken.jp.

Funding

This project was financially supported by research grants from RIKEN Brain Science Institute; the Ministry of Education, Sports, Science and Technology; the Ministry of Health, Labour and Welfare; grants-in-aid for the Molecular Imaging Program; Japan Science and Technology Agency Precursory Research for Embryonic Science and Technology; the Cell Science Research Foundation and the Takeda Science Foundation.

Notes

The authors declare the following competing financial interest(s): Takaomi C Saido and Takashi Saito hold rights under a United States patent entitled Model mouse of Alzheimer's disease expressing FAD APP 716 and use thereof. The patent number is 7,745,688. The authors, however, will provide academia with the mutant mice free of charge.

ACKNOWLEDGMENTS

We acknowledge the following members of the PNS lab: Yukio Matsuba, Naomi Mihira, Ryo Fujioka, Emi Hoseki, and Yuya Tomita for their technical assistance of the study. We are grateful for the support from Jiro Takano, Nobuhisa Iwata, and

Shigeyoshi Itohara. We thank Lars Tjernberg for helpful discussions during the preparation of this manuscript.

REFERENCES

(1) Hardy, J., and Selkoe, D. J. (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science 297*, 353−356.

(2) Jonsson, T., Atwal, J. K., Steinberg, S., Snaedal, J., Jonsson, P. V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., Hoyte, K., Gustafson, A., Liu, Y., Lu, Y., Bhangale, T., Graham, R. R., Huttenlocher, J., Bjornsdottir, G., Andreassen, O. A., Jonsson, E. G., Palotie, A., Behrens, T. W., Magnusson, O. T., Kong, A., Thorsteinsdottir, U., Watts, R. J., and Stefansson, K. (2012) A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature 488*, 96−99.

(3) Saito, T., Matsuba, Y., Mihira, N., Takano, J., Nilsson, P., Itohara, S., Iwata, N., and Saido, T. C. (2014) Single App knock-in mouse models of Alzheimer's disease. *Nat. Neurosci. 17*, 661−663.

(4) Welander, H., Frånberg, J., Graff, C., Sundström, E., Winblad, B., and Tjernberg, L. O. (2009) Aβ43 is more frequent than Aβ40 in amyloid plaque cores from Alzheimer disease brains. *J. Neurochem. 110*, 697−706.

(5) Higuchi, M., Iwata, N., Matsuba, Y., Takano, J., Suemoto, T., Maeda, J., Ji, B., Ono, M., Staufenbiel, M., Suhara, T., and Saido, T. C. (2012) Mechanistic involvement of the calpain-calpastatin system in Alzheimer neuropathology. *FASEB J. 26*, 1204−1217.