

# How would preclinical Alzheimer's disease (AD pathology) occur? An insight from a genomic instability mouse model

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More than 95% of Alzheimer's disease (AD) is late-onset, in which patients show clinical cognition/behavior symptoms after age 65. Unlike early-onset AD that comes with mutations in genes directly involved in amyloid metabolism (APP, PSEN), genetic predispositions associated with late-onset AD are harder to pinpoint, and their mechanistic links to AD development need further investigation. Although the development mechanism of late-onset AD remains controversial, amyloid-beta accumulation, initiated in middle age, is widely accepted as the triggering event for early AD pathology (Du Bois et al., 2010; Sterling et al., 2011). In 2018, we reported a genomic instability mouse model (Sgo1<sup>-/-</sup>) in which amyloid-beta accumulates in the brain in old age without early-onset AD mutation (Rao et al., 2018). The identification led us to anticipate that the model may reveal the development mechanism of late-onset AD. In a new study that appeared in *Aging Cell* (Rao et al., 2020a), we identified GSK3 inactivation in middle age as a cause for triggering the amyloid-beta accumulation. Inactivation of GSK3 appeared to affect amyloid-beta generation in two ways: (i) via increasing ARC/Arg3.1, which can generate amyloid-beta in an activity-dependent manner, and (ii) via activating canonical Wnt signaling and driving the cell cycle in the brain, thus activating the "amyloid-beta accumulation cycle" (Rao et al., 2020b). Since the Sgo1<sup>-/-</sup> condition prolongs mitosis, during which amyloid-beta generation and accumulation is facilitated, Sgo1<sup>-/-</sup> may be mimicking the aneuploid condition prevalent in patients with mild cognitive impairment (MCI) and AD (Potter et al., 2019). Our studies (a) support the notion of the critical roles of genomic instability and aneuploidy in AD development, (b) suggest a role of GSK3 in the onset of amyloid-beta accumulation and onset of AD pathology in middle age, and (c) suggest the usefulness of the mouse model for testing drug candidates for late-onset AD.

**Late-onset Alzheimer's disease (AD) development in the early asymptomatic phase:** Late-onset AD is a lethal disease for which we lack full mechanistic understanding and therapeutic/disease-

modifying medicine (Long, Holzman, 2019). With an increasing number of AD patients predicted, investigations for a mechanistic understanding and development of intervention or therapy remain crucial.

Brains of patients with late-onset AD with clinical cognition-behavior symptoms indicate signs of neurodegeneration and various histopathological changes. The major histopathological traits of clinical late-onset AD are (a) amyloid-beta plaques, (b) neurofibrillary tangles made of phosphorylated-TAU/TAU, and (c) cerebral amyloid angiopathy.

Yet, the late-stage AD pathologies do not appear overnight. Late-onset AD development is progressive and takes many years to fully manifest. Accumulation of amyloid-beta in the brain, beginning in middle age, is considered a triggering event for other late-onset AD pathologies. Amyloid-beta accumulation precedes other histopathological changes (including neurofibrillary tangles) and clinical cognition-behavior symptoms by 10–15 years (Sterling et al., 2011; Long and Holzman, 2019). The early/onset asymptomatic phases of late-onset AD have been recognized by broader medical communities. American Alzheimer's association uses the term "Preclinical AD," while French groups use "Pathological AD" for normal and functioning people with accumulated amyloid-beta (Du Bois et al., 2010; Sterling et al., 2011).

## What triggers amyloid-beta accumulation in middle age with late-onset AD?

Patients with early-onset AD carry genetic mutation(s) in genes directly involved in amyloid metabolism [i.e., amyloid precursor protein (APP), presenilin (PSEN)]. Genetic predispositions for late-onset AD, TREM2 and APOE4 subtype, are also involved in amyloid-beta clearance (Uddin et al., 2019; Liu et al., 2020). Thus, amyloid-beta metabolism seems critical in developing amyloid-beta accumulation. Is amyloid-beta accumulation beginning in middle age triggered by an age-associated change in amyloid-beta metabolism? It seems likely. But, demonstrating the causal link between the "change" and amyloid metabolism is tricky. There are numerous age-associated changes

that may not affect amyloid metabolism; in addition, quantifying age-associated changes and/or amyloid metabolism can present technical difficulties.

A majority of late-onset AD patients do not carry mutation in early-onset AD genes, suggesting that other factors not directly involved in amyloid metabolism may be involved in late-onset AD (e.g., Arango et al., 2001). As such, how/why amyloid-beta begins to accumulate in middle-aged "normal"-looking people is a mystery that has not been addressed nor elucidated (**Figure 1A**). Once the mystery is solved, we should be able to intelligently design AD intervention, and the approach may be different from existing approaches that have proven ineffective.

**A mouse model with genomic instability shows amyloid-beta accumulation in the brain, beginning in late-middle age:** We have been investigating effects of genomic instability in the body, using transgenic genomic instability mouse models. These models were originally developed to investigate the relationship between genomic instability and cancer. The mouse models were also anticipated to be useful for investigations of other biological roles of genomic instability *in vivo*. For example, genomic instability is a hallmark of aging (López-Otín et al., 2013). Not only an indicator of aging, genomic instability can also be a cause of aging, as suggested by the BubR1-hypomorphic genomic instability mouse model showing a progeric phenotype (Baker et al., 2011). Genomic instability is suggested to be involved in age-associated diseases.

Will genomic instability mouse models show any age-associated diseases or pathology? In part due to multiple disciplines and the long time required, this straightforward question was not experimentally addressed previously. As a high degree of aneuploidy and genomic instability is associated with MCI and AD (Potter et al., 2019), we hypothesized that genomic instability mice may show AD-related pathology and, in that case, they may serve as a model of sporadic late-onset AD. Indeed, we observed accumulated amyloid-beta in aged Sgo1<sup>-/-</sup> model mice, but not in aged BubR1<sup>-/-</sup> model mice. Focusing on the difference in the type of genomic instability in these two models (a major difference is the spindle checkpoint function), we suspected that spindle checkpoint activation and prolonged mitosis, observed in Sgo1<sup>-/-</sup> but not in BubR1<sup>-/-</sup>, are crucial for the amyloid-beta accumulation (Rao et al., 2018, 2020b). Amyloid-beta production is facilitated during mitosis, and prolonged mitosis in the Sgo1<sup>-/-</sup> model may

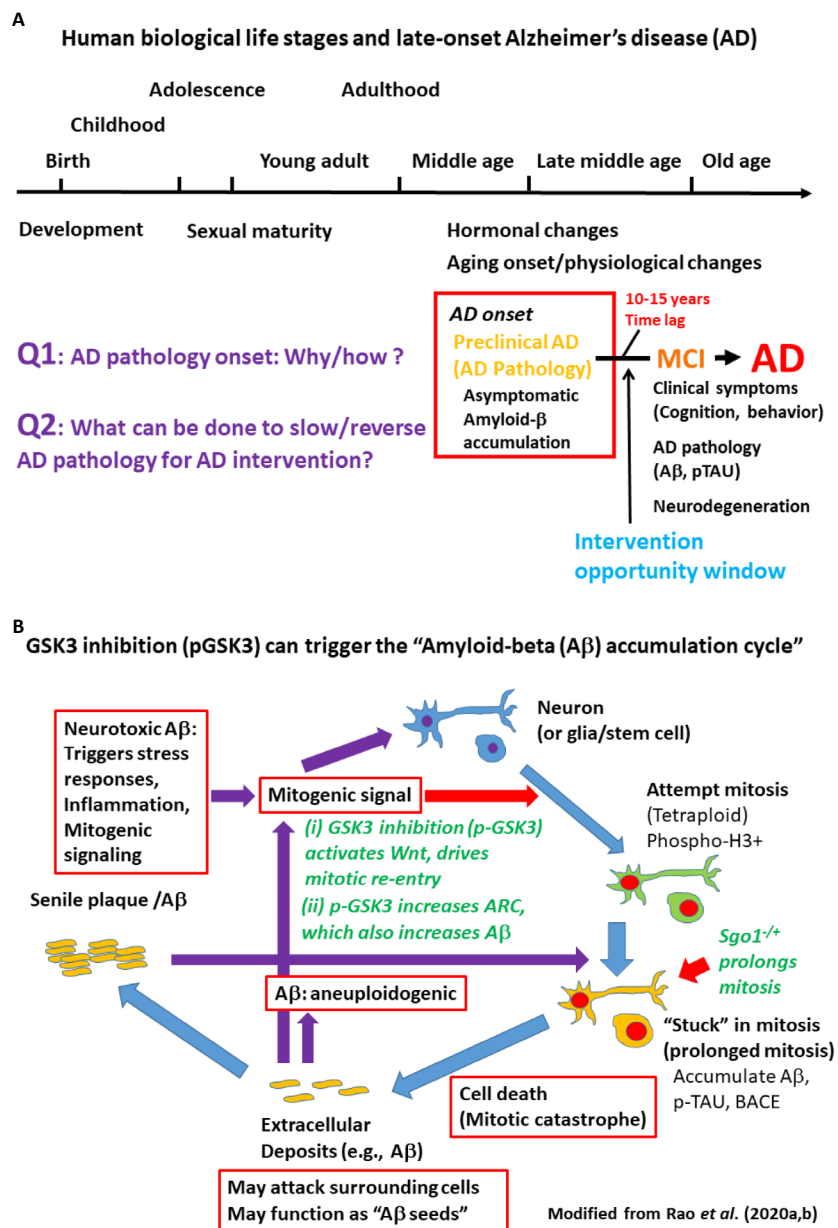
mimic or exaggerate high genomic instability and aneuploidy condition in aging brain. Aneuploid cells show characteristics that may contribute to amyloid-beta accumulation, including proteotoxic stress, transcriptomic alterations, and additional mitotic errors. In addition to amyloid-beta, aged *Sgo1*<sup>-/-</sup> indicated neuroinflammation and mis-regulated genes similar to human AD, recapitulating human AD pathology in other aspects (Rao et al., 2018, 2020a).

**The “amyloid-beta accumulation cycle” hypothesis:** The notion of a critical role of prolonged mitosis in late-onset amyloid-beta accumulation was combined with unique characteristics of amyloid-beta (i.e., oligomeric, neurotoxic, inflammagenic, mitogenic, aneuploidogenic) and observed signs of mitotic re-entry of neurons in AD patients. The combined concepts led us to an integrated hypothesis, the “amyloid-beta accumulation cycle” (Rao et al., 2020b; **Figure 1B**).

The “amyloid-beta accumulation cycle” hypothesis purports the occurrence of vicious cycles of events leading to amyloid-beta accumulation: initial increase in amyloid-beta; growth/mitogenic signaling activation and inflammation triggered by the amyloid-beta exposure, cell cycle activation, and mitotic re-entry; accumulation of amyloid-beta during (quasi-)mitotic state; mitotic catastrophe; and release of more amyloid-beta from dead cells into the microenvironment, which leads to another cycle. The sequence of events is well-corroborated with previous studies and observations (Potter et al., 2019, Rao et al., 2020b). Currently, it is our main working hypothesis.

**GSK3 inactivation in the middle-aged *Sgo1*<sup>-/-</sup> mouse, and its implications:** There are still many points to be investigated in the “amyloid-beta accumulation cycle.” After observing the timing of amyloid-beta accumulation in *Sgo1*<sup>-/-</sup> mice (late middle age; 15–18 months of age), we focused our attention on the supposed mitogenic signaling involved in the *Sgo1*<sup>-/-</sup> mouse model. After testing growth signaling of interest (including the cGAS/STING pathway, which was negative/did not seem to be activated), our unexpected finding was GSK3 inactivation with inhibitory phosphorylation on GSK3 $\alpha$ -S21 and GSK3 $\beta$ -S9. GSK3 inactivation can trigger (a) canonical Wnt signaling toward mitotic re-entry and (b) accumulation of ARC/Arg3.1, which can increase amyloid-beta in an activity-dependent manner (Rao et al., 2020a).

GSK3 inactivation was unexpected, because GSK3 is known to drive some AD pathologies,



**Figure 1 | Unsolved questions and a hypothesis on AD initiation/development (“the Amyloid-beta accumulation cycle”).**

(A) Late-onset AD is triggered with amyloid-beta accumulation in the middle age. (Question 1) Why/how does AD pathology occur, and (Question 2) What can be done to slow/reverse AD pathology for AD intervention? remain as unsolved questions. Elucidation of the mechanism will allow us to intelligently design intervention methods and apply them during the intervention opportunity window. In our transgenic mouse-based studies, a type of genomic instability (cohesinopathy-mediated chromosome instability) resulted in late-onset amyloid-beta accumulation in the brain. The findings suggest that genomic instability and resulting aneuploidy with prolonged mitosis can trigger and facilitate amyloid-beta accumulation. As genomic instability is a hallmark of aging (López-Otín et al., 2013) that increases with age, the intervention opportunity window may open at a specific age. The intervention target may encompass various, yet-to-be-tested pathways, such as the “cell cycle”, “aneuploidy-tolerating” or “aneuploid-specific cell killing with mitotic stress modulation” pathways. (B) GSK3 inhibition (pGSK3) can trigger the “amyloid-beta accumulation cycle.” The “amyloid-beta accumulation cycle” is our current working hypothesis to explain how amyloid-beta is accumulated in the brain (see text). Modified from Rao et al. (2020a, b). Amyloid-beta production is facilitated during mitosis, and prolonged mitosis in the *Sgo1*<sup>-/-</sup> model may mimic or exaggerate high genomic instability and aneuploidy conditions in aging brains. In the brains of middle-aged *Sgo1*<sup>-/-</sup> mice, GSK3 is inhibited via phosphorylation. The GSK3 inhibition (i) activates Wnt signaling and drives mitotic re-entry, which can lead to further amyloid-beta generation during (prolonged) mitosis, mitotic catastrophe, amyloid-beta release to the microenvironment, and the next cycle of the “Amyloid-beta accumulation cycle.” The GSK3 inhibition also (ii) shuts down GSK3-mediated proteasomal degradation of ARC and increases the amount of ARC. ARC can generate amyloid-beta in an activity-dependent manner. Thus, GSK3 inhibition can trigger amyloid-beta accumulation in middle age. AD: Alzheimer’s disease.

especially pTAU-based neurofibrillary tangles and neurodegeneration. GSK3 inhibitors have long been proposed to be used for AD therapy.

At this moment, we think the role of GSK3 in triggering amyloid-beta accumulation in middle age and the role in driving late-stage AD pathology are independent, different aspects of GSK3 functions. The observation may partly explain why we have not observed extensive pTAU neurofibrillary tangles pathology in *Sgo1<sup>-/-</sup>* mice, as GSK3 is one of the TAU kinases. We further speculate that after a certain time of amyloid-beta accumulation with inhibited GSK3, there may be a tipping point or a threshold, beyond which GSK3 is activated and neurofibrillary tangles begin to manifest.

Our observation supports the notion that inactivation of GSK3 is a trigger for the “amyloid-beta accumulation cycle” in middle age, initializing AD pathology. It also suggests that we may need to be careful with using GSK3 inhibitors in middle age for AD “intervention.” Indeed, clinical reports suggest that bipolar patients treated with lithium salts (a GSK3 inhibitor) may have a higher rate of AD later in life. The reports suggest that undue inhibition of GSK3 may indeed trigger and facilitate early AD pathology, although interpreting the complex biology of bipolar disease and AD requires caution.

**Prospective toward late-onset AD intervention: both clinical studies on “preclinical AD” people and mouse model-based study are needed:** Whether the triggering of AD pathology occurring with GSK3 inactivation in human preclinical AD patients remains to be tested. In clinical settings, we will face technical challenges of defining and identifying preclinical AD patients and procuring samples. Thus, we are attempting to gain additional insights from ongoing mouse model-based studies.

Most existing AD mouse models are based on transgenic manipulation of early-onset AD-associated genes (i.e., APP, PSEN1, PSEN2) with or without overexpression or humanization. Examples include 3xTG, 5xFAD, APP knock in, and APP<sub>swe</sub>/PS1. As mechanisms of onset and development of late-onset AD remain unclear, the AD modeling inevitably has focused on amyloid metabolism and early-onset AD. As such, existing AD modeling may have missed out some aspects of AD development from preclinical drug research and development process. With serendipity, here discovered is the *Sgo1<sup>-/-</sup>* genomic instability mouse model that shows late-onset AD pathology

without early-onset AD gene mutation. We are hoping that the model reveals mysteries of late-onset AD onset and development, as well as effective methods to intervene in and/or cure late-onset AD.

*Our recent work relevant to this prospective were supported by the Kerley-Cade Chair fund to CVR; and by research funds from the Stephenson Cancer Center and by a Pilot Project Award from the Oklahoma Nathan Shock Center for Excellence in the Basic Biology of Aging to HYY.*

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**Date of submission:** October 23, 2020

**Date of decision:** December 4, 2020

**Date of acceptance:** December 18, 2020

**Date of web publication:** February 19, 2021

<https://doi.org/10.4103/1673-5374.308096>

**How to cite this article:** Rao CV, Yamada HY (2021) How would preclinical Alzheimer's disease (AD pathology) occur? An insight from a genomic instability mouse model. *Neural Regen Res* 16(10):2012-2014.

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*C-Editors: Zhao M, Song LP; T-Editor: Jia Y*