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Original Article

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FAM19A5 Deficiency Mitigates the Aβ Plaque Burden and Improves Cognition in Mouse Models of Alzheimer's Disease

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FAM19A5, a novel secretory protein highly expressed in the brain, is potentially associated with the progression of Alzheimer's disease (AD). However, its role in the AD pathogenesis remains unclear. Here, we investigated the potential function of FAM19A5 in the context of AD. We generated APP/PS1 mice with partial FAM19A5 deficiency, termed APP/PS1/FAM19A5$^{+/LacZ}$ mice. Compared with control APP/PS1 mice, APP/PS1/FAM19A5$^{+/LacZ}$ mice exhibited significantly lower Aβ plaque density and prolonged the lifespan of the APP/PS1 mice. To further explore the therapeutic potential of targeting FAM19A5, we developed a FAM19A5 antibody. Administration of this antibody to APP/PS1 mice significantly improved their performance in the Y-maze and passive avoidance tests, indicating enhanced cognitive function. This effect was replicated in 5XFAD mice, a model of early-onset AD characterized by rapid Aβ accumulation. Additionally, FAM19A5 antibody treatment in 5XFAD mice led to enhanced exploration of novel objects and increased spontaneous alternation behavior in the novel object recognition and Y-maze tests, respectively, indicating improved cognitive function. These findings suggest that FAM19A5 plays a significant role in AD pathology and that targeting with FAM19A5 antibodies may be a promising therapeutic strategy for AD.

Key words: FAM19A5, Alzheimer disease, Aβ plaque, Cognitive function

INTRODUCTION

Alzheimer's disease (AD) is characterized by the accumulation of amyloid-beta (Aβ) forming plaques in the brain. These plaques are central to the pathogenesis of AD, leading to neuronal damage and cognitive decline [1]. Consequently, therapeutic strategies targeting the clearance of Aβ plaques have emerged as a major focus in AD research. These approaches, including anti-amyloid immunotherapies, have shown promise in reducing the Aβ burden in the brain and delaying disease progression [2]. However, clinical trials of anti-amyloid therapies have shown their limited effectiveness against cognitive decline, raising concerns about their overall efficacy [3]. Therefore, identifying the ideal target to reverse cognitive decline would be crucial for therapeutic intervention and represents a promising new direction for AD research [4].

FAM19A5, a recently discovered brain-specific protein [5], has emerged as a potential player in both brain development and neurological diseases. Intriguingly, FAM19A5 exhibits a highly localized expression pattern, particularly concentrated in layers 2/3 and 5 of the cortex and CA regions of the hippocampus during critical stages of growth [6]. This strategic positioning suggests a role in memory, learning, or other hippocampal functions. Furthermore, recent studies linked FAM19A5 to neurological disorders such as AD, raising the possibility that FAM19A5 level or function contributes to disease pathology [7-10].

Further strengthening this connection, a recent study identified FAM19A5 as a key regulator of synaptic elimination, a process implicated in AD progression. Overexpression of the FAM19A5 gene

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in mouse hippocampal neurons, as well as treating the neurons with FAM19A5, both resulted in a significant reduction in the density of spines that are in contact with presynaptic nerve terminals. However, neutralizing FAM19A5 with a FAM19A5 antibody effectively reversed this effect, demonstrating the crucial role of FAM19A5 in regulating spine density and synapse assembly [11]. Given the established link between synaptic loss and cognitive decline in AD [12], these findings suggest that inhibiting FAM19A5 may offer a novel therapeutic strategy to mitigate synaptic degeneration and cognitive impairment. However, despite understanding FAM19A5's involvement in synaptic regulation, the precise mechanisms underlying its role in AD pathogenesis remain to be fully elucidated. This knowledge may be essential for optimizing therapeutic approaches for patients with AD.

In this study, we investigated the role of FAM19A5 in AD progression. We generated mice with partial FAM19A5 knockout using the LacZ knock-in technique [6, 13]. These mice were then crossbred with a mouse model of AD to study the association between FAM19A5 and amyloid plaque accumulation. We found that AD mice crossbred with FAM19A5 LacZ KI mice exhibited a reduced amyloid plaque burden and extended the lifespan of mice. To develop a potential therapeutic approach, we further generated FAM19A5 antibodies to target the FAM19A5 protein in the brain. Administration of these antibodies to mouse models of AD resulted in improved cognitive performance. These results suggest that FAM19A5 could be a promising novel target for therapeutic intervention in AD.

MATERIALS AND METHODS

Animals

Double-transgenic mice (B6. Cg-Tg [APPswe, PSEN1dE9] 85Dbo/J) (stock #004462) was obtained from the Jackson Laboratory (Bar Harbor, ME, USA). These mice coexpress the PS1△dE9 mutant form of PS1 and a chimeric mouse-human APP695 strain harboring the Swedish K594N and M595L mutations. Two transgenic genes are controlled by independent mouse prion protein promoter elements, which drive high protein expression in neurons and astrocytes of the CNS.

5XFAD transgenic mice, B6. Cg-Tg (APPSwFlLon PSEN1* M146L*L286V) 6799 Vas/Mmjax was obtained from the Korea Institute of Brain Science. Transgenic mice with mutant human APP (695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) familial Alzheimer's disease (FAD) mutations and human PS1 harboring two FAD mutations, M146L and L286V, were generated. The 5XFAD mice accumulate a high level of amyloid deposition at approximately 1.5 months of age, and plaques spread throughout the hippocampus and cortex by 6 months of age.

The FAM19A5 LacZKI used in this study was generated by the UC Davis Mouse Biology Program (MBP). These transgenic mice were derived from in-house breeding colonies backcrossed onto a C57BL/6 background. For this study, a new crossbred mouse model, APP/PS1/A5LacZ, was generated by crossbreeding APP/PS1 mice with FAM19A5 LacZ heterozygous mice. The genotypes of all the mice were determined by PCR using tail genomic DNA. PCR genotyping was carried out with the following two primers: All the animals were housed under a 12-12 h light-dark cycle (light phase, 8:00 A.M. to 8:00 P.M.) with standard laboratory diet and water available ad libitum. All animal handling and experiments were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) of Korea University Medical School.

Production of anti-FAM19A5 antibody

We generated a chimeric chicken/human monoclonal antibody named 3-2 against FAM19A5 by immunizing chickens with purified recombinant FAM19A5 via a previously described method [13].

Antibody administration

APP/PS1 and 5XFAD mice were treated with an FAM19A5 antibody or human IgG as a control according to different protocols. To assess FAM19A5 antibody and FAM19A5 levels in plasma, 14-month-old male APP/PS1 mice were treated with a single IV administration of an FAM19A5 antibody (10 mg/kg). Then, plasma and brain samples were collected to measure the FAM19A5 and FAM19A5 antibody levels. To test the cognition improvement in APP/PS1 mice, 8-month-old male APP/PS1 mice were treated IV with a FAM19A5 antibody (5 mg/kg) or hIgG (5 mg/kg) four times weekly.

To test the cognition improvement in 5XFAD mice following antivody treatment, 4-month-old 5XFAD mice were treated IV with a FAM19A5 antibody (5 mg/kg) or hIgG (5 mg/kg) twice a month (every 15 days) and sacrificed after the NOR test. Additionally, 4-month-old 5XFAD mice were treated intravenously with a FAM19A5 antibody (2.5 mg/kg) or hIgG (2.5 mg/kg) four times a month (every 7 days) and were subjected to the Y-maze test.

Thioflavin-S staining and plaque quantification

To identify Aβ plaques, brain sections from 11-month-old APP/PS1 and APP/PS1/FAM195 LacZ mice were stained with thioflavin-S solution. The sections were treated with 1% thioflavin-S solution for 10 min and then destained in 70% alcohol for 3 min.

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Images were acquired with a confocal laser scanning microscope (Leica TCS SP8). Five to eight sections of each transgenic brain were selected for quantification of amyloid plaques using ImageJ software (NIH).

ELISA

To assess Aβ levels, cortical and hippocampal tissues were homogenized in 0.5 ml of Tris-buffered saline (TBS) containing 25 mM Tris-HCl (pH 7.4), 130 mM NaCl, 2.7 mM KCl, 5 mM EDTA, and a protease inhibitor cocktail (Thermo), using a Polytron benchtop lab homogenizer (Wheaton) at 4°C. The homogenate was then centrifuged at 14,000 rpm for 30 minutes at 4°C. The supernatants were collected and stored at -80°C for further analysis. The remaining pellet was sonicated in 0.5 ml of 2% SDS in TBS on ice, followed by centrifugation at 14,000 rpm for 40 minutes at 4°C. The resulting supernatants, representing the soluble fraction, were collected for the analysis of SDS-soluble Aβ. The pellet was then resuspended and sonicated in 0.5 ml of 70% formic acid, followed by another centrifugation at 14,000 rpm for 40 minutes at 4°C. The formic acid supernatants, referred to as the insoluble fraction, were neutralized with a 1:20 dilution in 1 M Tris-phosphate buffer (pH 11.0) and used to measure formic acid-soluble Aβ. Aβ (1-42) levels were quantified using ELISA kits from R&D Systems.

To measure FAM19A5 in biofluids and tissues, we immobilized the LRRC4B (453-576) protein onto 96-well microplates. The proteins were diluted in 50 mM carbonate buffer (pH 9.6) to a final concentration of 1 μg/ml and incubated overnight at 4°C. After being washed with PBS containing 0.05% Tween 20, the plates were blocked with a blocking buffer (PBS with 1% BSA and 0.05% Tween 20) for 1 hour at 37°C. Standards and samples were added to the wells and incubated for 90 minutes at room temperature (22~25°C). Following additional washes, a HRP-conjugated FAM19A5 antibody was added and the samples were incubated at 37°C for 1 hour. TMB substrate was then added, and the colorimetric reaction was stopped with sulfuric acid. The absorbance was measured at 450 nm using a microplate reader.

To measure CSF NS101, we used a pair of rabbit anti-human IgG heavy chain antibodies and HRP-conjugated goat anti-human IgG kappa light chain antibodies.

Novel object recognition (NOR)

The object recognition test was performed as previously described [18]. In the habituation phase, the mice were allowed to explore the open field (30 cm×30 cm×30 cm) for 5 minutes twice a day at 6-hour intervals for 3 days. On the 4th day, the training phase, in which the mice were placed in the same open field but with two identical objects placed 5 cm from the wall of the open field, was initiated. The mice were placed in such a way that their heads were opposite to the objects, and then video was recorded for 10 minutes. After 6 hours, the test session was performed. This phase is similar to the training session except that one of the familiar objects is replaced by a new or novel object. During all three phases, both the open field and the objects were cleaned with 70% ethanol and dried before use to minimize error due to olfactory cues. This test was analyzed by using ANY-maze behavioral tracking software.

Y-maze

The Y-maze is a behavioral test used for assessing memory function to test spontaneous alternation performance. This test utilizes a platform constructed of white, nonreflective plastic and consisting of three arms (arms A, B, and C) oriented at a 120° angle relative to each other with a central triangular area (mid zone). The animals were placed at the end of the first arm and then allowed to explore freely for 5 mins. The sequence and total number of arms entered were recorded. The percentage of correct alternations is the number of triads containing entries into all three arms/maximum possible alternations (the total number of arms entered -2) ×100. This test was analyzed by using ANY-maze behavioral tracking software.

Passive avoidance test

The mice were habituated to the fear conditioning chamber for 10 minutes without any stimulus. The next day, a training session was performed consisting of seven consecutive trials, each with a tone (5 kHz, 70 dB, 30 seconds) followed by a foot shock (0.7 mA, 2 seconds). The mice were returned to their home cages, and the test session was conducted the following day.

For the contextual memory test, the mice were placed in the same chamber for 5 minutes without tone or electric shock. Freezing time was analyzed using ANY-Maze 6.36 software (Stoelting). For the auditory memory test, the mice were placed in a distinct context and exposed to 3 tones without foot shocks, with a 90-second intertrial interval after a 5-minute acclimatization period. The freezing time during each tone was analyzed using ANY-Maze 6.36 software (Stoelting), and the average freezing time was calculated.

Statistical analysis

The data are expressed as the mean±SEM. The group means were compared using paired and unpaired Student's T-tests, one-way ANOVA or two-way ANOVA followed by Bonferroni's multiple comparison test. Statistical analysis was performed with GraphPad Prism 5.0 software. Statistical significance was defined as a p value less than 0.05. The results from the behavioral studies, quantitative

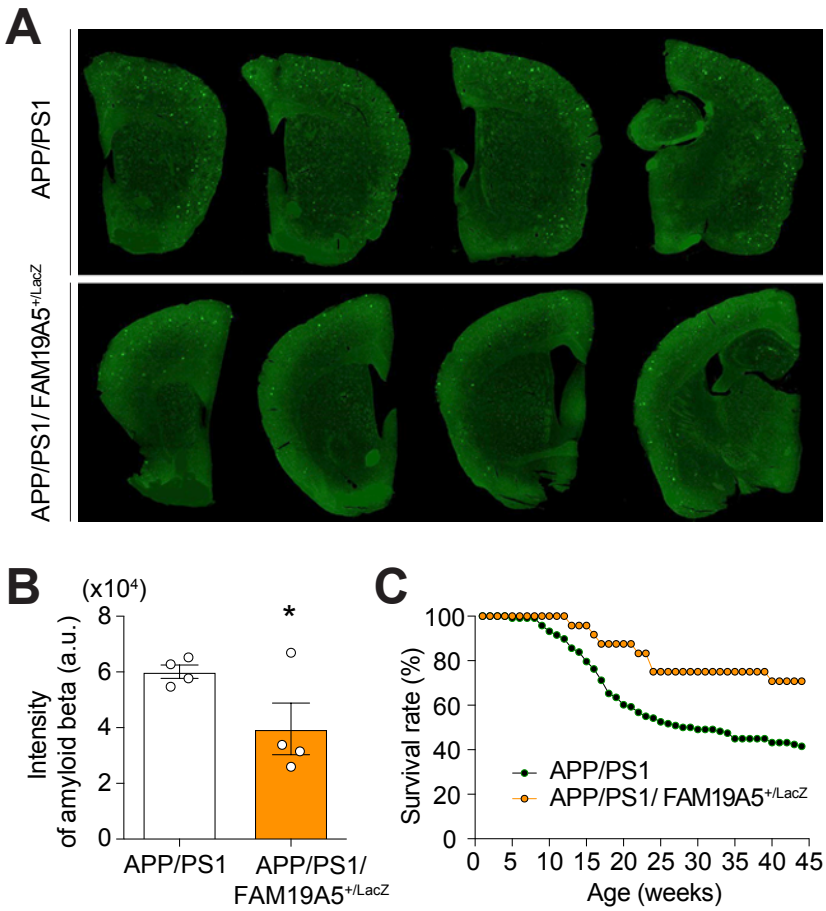

Fig. 1. Aβ plaque reduction and extended lifespan in APP/PS1/FAM19A5LacZ mice. (A) Thioflavin-S staining of brain slices to visualize amyloid plaques (green) and (B) their quantification. Values represent mean±SEM, n=4 each (11-month-old mice, 2 males, 2 females), unpaired T-test, *p<0.05. (C) Survival rate of APP/PS1 and APP/PS1/FAM19A5$^{+/LacZ}$ mice, n=118 (APP/PS1, 58 males, 60 females), 24 (APP/PS1/FAM19A5$^{+/LacZ}$, 12 males, 12 females).

immunoblot analyses and serum levels were statistically evaluated using Student's T-test, one-way ANOVA or two-way ANOVA followed by Bonferroni's multiple comparison test. Statistical analysis was performed with GraphPad Prism 5.0 software. Statistical significance was defined as a p value less than 0.05.

RESULTS

Effect of partial FAM19A5 knockout on APP/PS1 transgenic mice

Recent findings suggest that FAM19A5 levels in the brain are potentially correlated with neurodegenerative cascades, including AD [6, 9, 10]. We hypothesized that reducing FAM19A5 expression would have a therapeutic benefit for AD, leading to improvements in cognitive function. To test this, we crossbred AD mice with FAM19A5 LacZ knock-in (KI). FAM19A5 LacZ mice carry a LacZ reporter gene inserted before exon 4 of the FAM19A5 gene. Consequently, these mice have lower FAM19A5 levels in the brain than wild-type mice [14].

Crossbreeding FAM19A5 LacZ hemizygous mice with APP/PS1 mice generated WT, APP/PS1 and APP/PS1/FAM19A5$^{+/LacZ}$ mice. To investigate the Aβ plaque burden in the mice, brain tissues from 11-month-old APP/PS1 and APP/PS1/FAM19A5$^{+/LacZ}$ mice were stained with thioflavin-S (Fig. 1A). For image analysis, 6 brain slices from the olfactory bulb to the cerebellum were selected, and the intensity of the signals was measured. Although Aβ plaques were observed in the cortex and hippocampus of both genotypes, APP/PS1/FAM19A5$^{+/LacZ}$ mice exhibited a significantly lower Aβ plaque density than did APP/PS1 mice (Fig. 1B). APP/PS1/FAM19A5$^{+/LacZ}$ mice also showed an extended lifespan. We tracked the survival rates of APP/PS1 and APP/PS1/FAM19A5$^{+/LacZ}$ mice for 45 weeks. The survival rate of APP/PS1 mice began to decline at approximately 8 weeks of age, and only approximately 40% of the mice survived to 45 weeks. In contrast, the survival rate of APP/PS1/

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FAM19A5$^{+/LacZ}$ mice began to decline at approximately 13 weeks of age, and 65% of the mice survived to 45 weeks (Fig. 1C). This result may suggest that partial depletion of FAM19A5 could have a protective effect against the decline in survival rates typically observed in the progression of AD.

Systemic administration of the anti-FAM19A5 antibody facilitates the release of FAM19A5 from the brain in APP/PS1 mice

The therapeutic effects observed in APP/PS1/FAM19A5$^{+/LacZ}$ mice support the potential of targeting FAM19A5 as a novel therapeutic approach. To achieve specific targeting of FAM19A5 in the brain, we developed a FAM19A5 antibody that can be delivered to the brain [11]. The FAM19A5 antibody was administered intravenously (IV) as a single dose to 14-month-old male APP/PS1 mice. The levels of the antibody in both the plasma and brain were monitored over a period of 28 days. The plasma levels of the FAM19A5 antibody peaked immediately after administration and then gradually decreased, showing a half-life of 6.8 days at a dose of 10 mg/kg (Fig. 2A, black). Prolonged systemic exposure facilitated the delivery of the antibody across the blood-brain barrier (BBB). In the brain, the FAM19A5 antibody reached peak levels approximately 1.25 days (30 hours) after IV injection. The antibody concentration then declined gradually over the next 28 days, with a half-life of 17.3 days (Fig. 2A, red). Based on the area under the curve calculations for plasma (11,209 μg · hr/ml) and brain (291 μg · hr/g) contents of the FAM19A5 antibody, approximately 2.6% of the plasma antibody was shown to cross the BBB, which is consistent with results observed in other clinically used antibodies [15].

Additionally, we monitored the concentration of FAM19A5 in the plasma. Typically, FAM19A5 levels in plasma are extremely low, while its concentrations in the brain and CSF are significantly higher [11]. However, following a single administration of the antibody, plasma FAM19A5 levels exhibited a sharp increase within 1.25 days (30 hours) and then gradually declined over the course of 28 days (Fig. 2B). Notably, the plasma FAM19A5 profile closely mirrors that of the FAM19A5 antibody profile in the brain. These findings suggest that FAM19A5 may be released from the brain's extracellular space upon interaction with the antibody, leading to the observed plasma FAM19A5 levels. Therefore, the antibody-induced increase in plasma FAM19A5 levels likely reflects the depletion of FAM19A5 in the extracellular spaces of the brain.

To investigate the effects of systemic administration of the FAM19A5 antibody on cognitive behavior and amyloid burden in APP/PS1 mice, the FAM19A5 antibody was administered IV to 8-month-old male APP/PS1 mice weekly for four weeks. Behavioral tests, including the Y-maze and passive avoidance tests, were conducted over 14 days. Following these assessments, the animals were sacrificed to evaluate the amyloid aggregate levels in the brain and the FAM19A5 levels in the serum.

APP/PS1 mice treated with a human IgG antibody exhibited very low serum FAM19A5 levels, comparable to those in WT mice. In contrast, APP/PS1 mice treated with the FAM19A5 antibody showed significantly higher serum FAM19A5 levels compared to other groups (Fig. 2C). The FAM19A5 concentrations were consistent with the levels measured in plasma on day 14 after

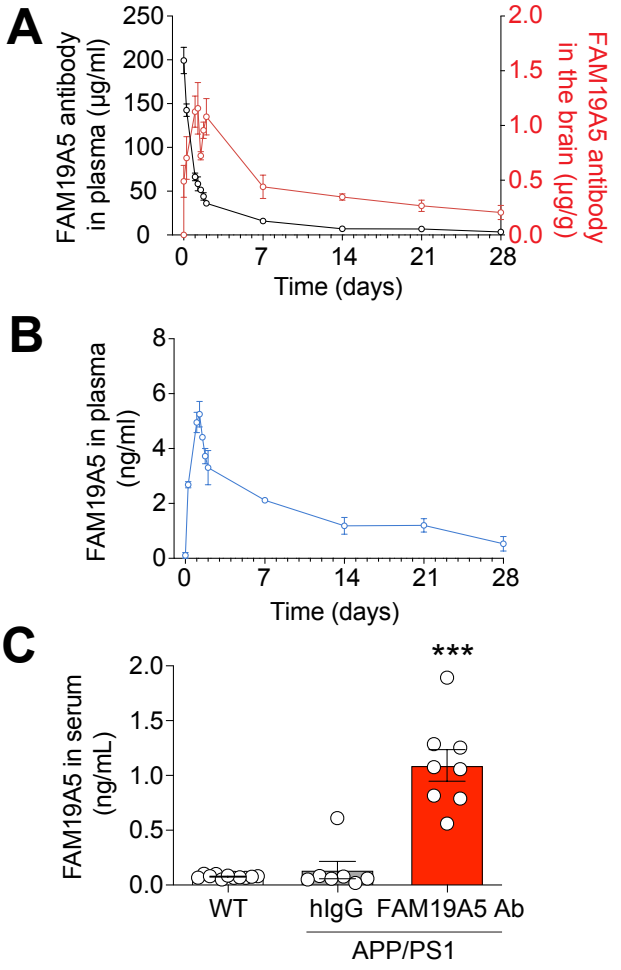

Fig. 2. FAM19A5 antibody-mediated FAM19A5 clearance in the brain. (A) FAM19A5 antibody levels in plasma (black) and in the brain (red) of 14-month-old male APP/PS1 mice at the indicated time points after a single IV administration of 10 mg/kg antibody (black), n=3 each (B) Plasma FAM19A5 levels in the APP/PS1 mice after a single IV administration of 10 mg/kg anti-FAM19A5 antibody, n=3 each (C) FAM19A5 levels in serum from 8-month-old male APP/PS1 mice, 14 days after receving four weekly IV administrations of a 5 mg/kg FAM19A5 or human IgG antibody. Data are presented as the mean±SEM. n=7~11, one-way ANOVA followed by Tukey's multiple comparison test, ***p<0.001 (FAM19A5 antibody vs. WT or hIgG).

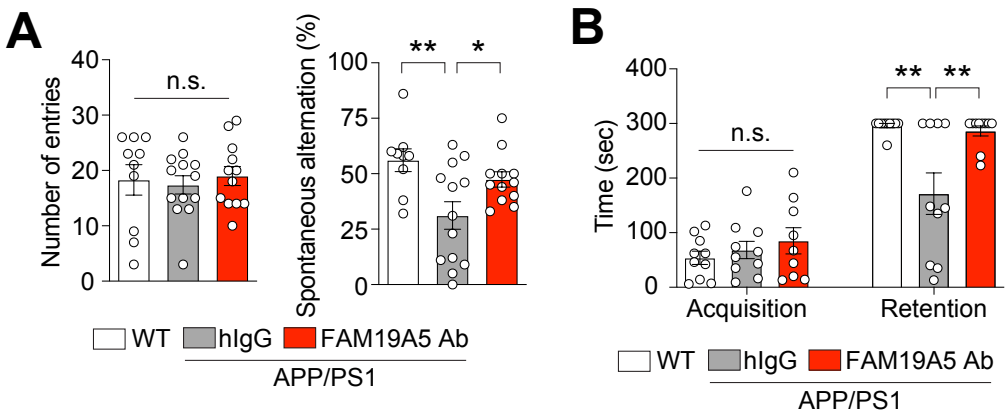

Fig. 3. Cognitive improvement in APP/PS1 following anti-FAM19A5 antibody treatment. Quantification of (A) total arm entries and spontaneous alternation in Y-maze after hIgG or anti-FAM19A5 antibody treatment in 8-month-old APP/PS1 mice, n=9~13 (male only), unpaired T-test, *p=0.0344, **p=0.0087. (B) Quantification of acquisition and retention times in the passive avoidance test following anti-FAM19A5 antibody treatment in 8-months-old APP/PS1 mice, n=9~10 (male only), one-way ANOVA followed by Tukey's multiple comparison test, **p=0.0037 (hIgG vs. FAM19A5 Ab). All values represent means±SEM.

the single administration (Fig. 2B), suggesting that the FAM19A5 antibody continuously induced the release of FAM19A5 secreted from the brain.

Effect of the anti-FAM19A5 antibody on cognitive behavior and Aβ aggregate burden in APP/PS1 mice

APP/PS1 mice that received four weekly injections of the FAM19A5 antibody were subjected to a Y-maze test to assess improvements in cognitive and memory function. Only male mice were used for the behavioral studies to minimize the potential confounding effects of hormonal fluctuations. Both wild-type and APP/PS1 mice exhibited robust exploration of all arms in the Y-maze. There was no significant difference in the total number of arm entries between the wild-type and APP/PS1 mice (Fig. 3A, left). Furthermore, within the APP/PS1 group, there were no significant differences in total arm entries between mice injected with human IgG (hIgG) antibodies and those injected with FAM19A5 antibodies, indicating that motor activity was preserved in all experimental groups. However, while APP/PS1 mice injected with hIgG showed a significant decrease in spontaneous alternation compared with wild-type mice, suggesting cognitive impairment, APP/PS1 mice treated with FAM19A5 antibodies exhibited a significant increase in spontaneous alternation relative to the hIgG group (Fig. 3A, right). Similar enhancements in cognitive memory were observed in the passive avoidance test. In this paradigm, memory acquired during the acquisition period was shown to be maintained at near wild-type levels. When the test resumed 24 hours later, APP/PS1 mice treated with hIgG exhibited significantly reduced memory compared to wild-type mice. In contrast, APP/PS1 mice treated with the FAM19A5 antibody displayed memory retention comparable to that of wild-type mice (Fig. 3B). These findings demonstrate that FAM19A5 antibodies can ameliorate the cognitive deficits associated with AD.

Next, we explored the impact of FAM19A5 antibody treatment on the Aβ aggregate burden in the brain. Following the behavioral tests, the mice were sacrificed, and ELISA was used to assess changes in amyloid oligomers and plaques in soluble and insoluble brain fractions, respectively. While a trend toward decreased Aβ oligomer and plaque levels was observed in the APP/PS1 mice following FAM19A5 antibody administration, this trend did not achieve statistical significance (Fig. 4).

Effect of the anti-FAM19A5 antibody on cognitive behavior in 5XFAD mice

Given the rapid Aβ accumulation characteristic of the 5XFAD model [16], we employed these mice to assess therapeutic efficacy in a model of early-onset AD. hIgG-treated 5XFAD mice did not exhibit a preference for the novel object in the NOR test, whereas FAM19A5 antibody treatment enhanced exploration of the novel object (Fig. 5A). Across the 3 habituation days, the activity of all the mice in the NOR test decreased, as indicated by a main effect of day, suggesting successful habituation. The total distance traveled did not differ among the experimental groups (Fig. 5B).

We further assessed spontaneous alternation performance in 5XFAD mice via the Y-maze test. The Y-maze test assesses spatial working memory by measuring the spontaneous alternation rate, which refers to the tendency of a mouse to explore a novel arm on each entry. A lower alternation rate is indicative of cognitive im-

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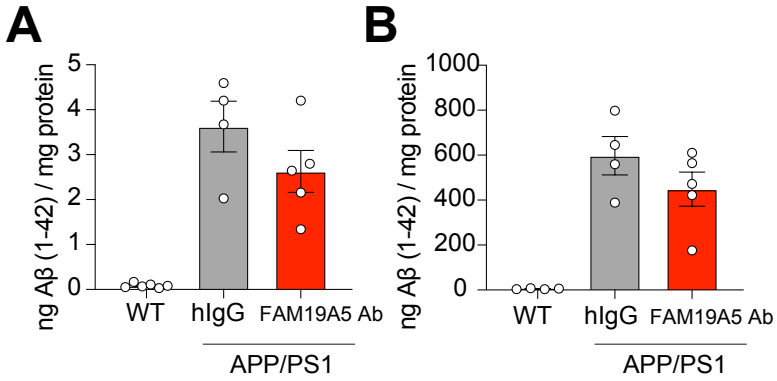

Fig. 4. Change in amyloid aggregates in APP/PS1 mice following anti-FAM19A5 antibody treatment. Quantification of amyloid aggregates from (A) the soluble and (B) insoluble brain fraction, respectively, following anti-FAM19A5 antibody administration to 8-month-old APP/PS1 mice. Values represent means±SEM, n=4~6 (male only), unpaired T-test (hIgG vs. FAM19A5 antibody), p=0.2129 (soluble), 0.2336 (insoluble).

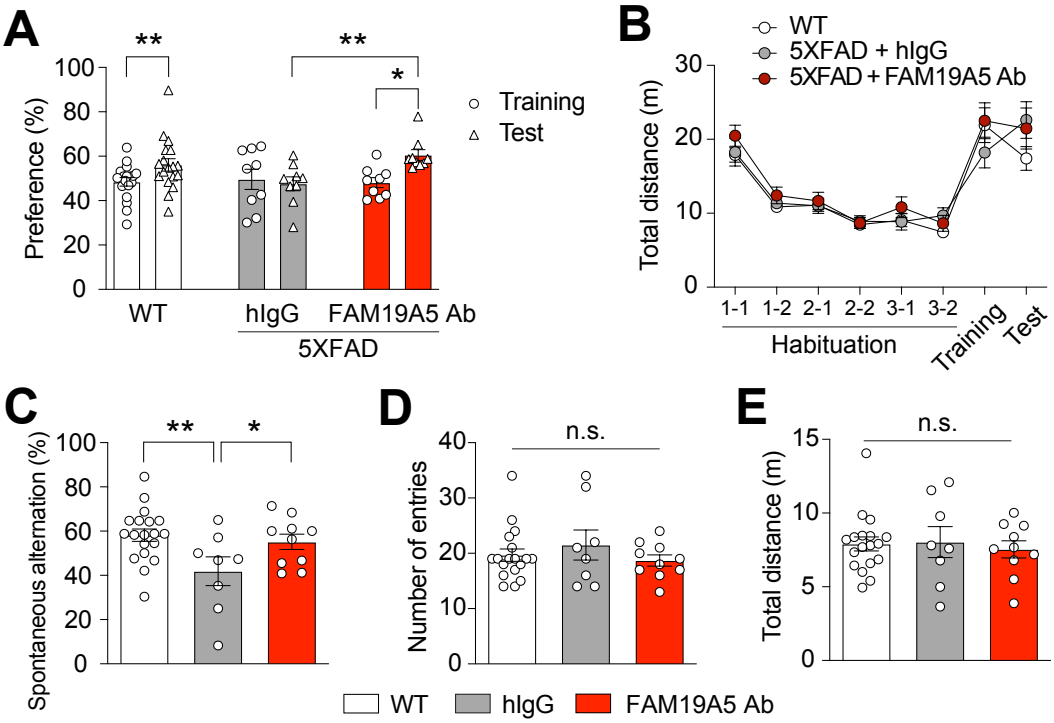

Fig. 5. Cognitive improvement in 5XFAD mice following anti-FAM19A5 antibody treatment. (A) Quantification of novel objective recognition after anti-FAM19A5 treatment in 4-month-old 5XFAD mice. (B) Total traveling distance in meters during the NOR test. n=8~18 (male only), unpaired T-test, *p<0.05, **p<0.01. (C) Spatial working memory of 3 or 4-month-old 5XFAD and WT mice was assessed by spontaneous alternation, (D) total number of arm entries, and (E) total distance in the Y-maze, respectively. Values represent means±SEM, n=8~18 (male only), unpaired T-test, *p<0.05, **p<0.01.

pairment. Compared with WT mice, 5XFAD mice exhibited significant cognitive impairment. However, compared with 5XFAD control mice, FAM19A5 antibody-treated 5XFAD mice exhibited a significant increase in spontaneous alternations (Fig. 5C). This improvement in exploratory behavior was comparable to that observed in WT mice. Notably, the number of total arm entries and total distance traveled within the maze did not differ between the groups, indicating that motor function and exploratory activity remained normal in 5XFAD mice (Fig. 5D, E).

DISCUSSION

In this study, we utilized two well-established AD mouse models, APP/PS1 and 5XFAD, to investigate therapeutic approaches tar-

geting amyloid plaque pathology. Although these models exhibit distinct amyloid plaque formation timelines, both effectively mimic early-stage AD. Transient administration of the FAM19A5 antibody resulted in a modest reduction in amyloid plaque burden while simultaneously improving cognitive function in both models by inhibiting the endogenous function of FAM19A5 in the brain. Given the efficacy and apparent long-term safety profile of this antibody [11], future studies are warranted to investigate the effects of chronic FAM19A5 antibody administration on amyloid plaque accumulation and cognitive function in late-stage AD models.

The present study employed well-established behavioral tests, the Y-maze, passive avoindance, and, the novel object recognition test to assess the cognitive benefits of FAM19A5 antibody treatment in APP/PS1 and 5XFAD mice. Notably, treatment with the antibody resulted in similar improvements in cognitive function in all these mouse models, as evidenced by their performance in these tasks. This observation is particularly significant because these models represent distinct stages of AD. The similar efficacy across models suggests that targeting FAM19A5 may be a disease-modifying therapeutic strategy, potentially offering benefits not only for symptomatic improvement but also for halting or slowing disease progression. This finding is especially promising considering the current challenges faced in clinical trials targeting amyloid beta plaques. Although these plaques are a hallmark pathology of AD, many such therapies have failed to translate into significant cognitive benefits in human patients [3]. Targeting FAM19A5 could represent a novel and more effective therapeutic approach for AD.

FAM19A5 antibody injection demonstrated a trend towards decreased amyloid aggregates, suggesting a direct or indirect involvement of FAM19A5 in amyloid aggregation. There are likely several reasons why the reduction in amyloid aggregation observed in APP/PS1 mice treated with the FAM19A5 antibody is less pronounced compared to those crossbred with FAM19A5 LacZ KI mice. First, the impact of FAM19A5 antibody treatment on amyloid aggregation is likely confined to the period during which the antibody is administered. In contrast, crossbreeding with FAM19A5 LacZ KI mice can influence the entire process of amyloid plaque formation, starting from the initial stages of amyloid production. Second, the FAM19A5 antibody primarily targets and removes extracellular FAM19A5 since it cannot penetrate cells [11]. However, crossbreeding with FAM19A5 LacZ KI mice may reduce FAM19A5 levels both extracellularly and intracellularly [14], thereby exerting a more comprehensive and potent effect on amyloid aggregation. This could explain why the reduction in amyloid aggregation is more pronounced in FAM19A5 LacZ KI mice.

Despite the lack of a significant reduction in amyloid plaques following transient FAM19A5 reduction via antibody treatment, we observed improvements in cognitive function. This finding aligns with recent work by Suzuki et al [17], which demonstrated that restoring synaptic function through synaptic organizer proteins can improve cognition in the presence of amyloid pathology. This finding implies that restoring synapses lost due to pathological factors in AD can be a crucial therapeutic strategy. This study further supports this notion by showing that neutralizing FAM19A5 leads to improved cognitive function. Therefore, the transient elimination of FAM19A5 through antibody treatment holds significant promise for the treatment of AD.

Intriguingly, our study revealed that APP/PS1 mice crossbred with FAM19A5 LacZ KI mice exhibited a significantly longer lifespan than wild-type APP/PS1 mice. This finding may suggest that a decrease in FAM19A5 can not only impact cognitive function but also influence overall disease progression in AD models. This observation strengthens the potential of FAM19A5 antibodies as a novel therapeutic strategy. Targeting FAM19A5 may not only achieve symptomatic improvements in cognitive function, as suggested by the electrophysiological and behavioral tests in AD mice [11], but also potentially modulate disease progression and extend the lifespan in AD patients. Further investigation is crucial to delineate the precise mechanisms by which FAM19A5 deficiency extends lifespan and to assess the efficacy of FAM19A5 antibodies in promoting longevity in preclinical models with advanced features of human AD pathology. Additionally, studies exploring the safety and efficacy of this approach in aged animals are warranted to inform its potential translation into clinical trials for AD patients.

CONFLICT OF INTEREST

SP, AS, ML, SML, and JYS are shareholders of Neuracle Science, Co., Ltd. The remaining authors have no conflicts of interest to declare.

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