Farnesyltransferase Haplodeficiency Reduces Neuropathology and Rescues Cognitive Function in a Mouse Model of Alzheimer Disease*

Received for publication, July 20, 2013, and in revised form, October 16, 2013 Published, JBC Papers in Press, October 17, 2013, DOI 10.1074/jbc.M113.503904

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Background: Protein prenylation may play an important role in Alzheimer disease. Results: Haplodeficiency in farnesyltransferase and geranylgeranyltransferase-1 attenuates neuropathology, but only reduction of farnesyltransferase rescues cognitive function in Alzheimer mice. Conclusion: Protein farnesylation and geranylgeranylation differentially affect the course of Alzheimer disease. Significance: Specific inhibition of protein farnesylation might be a potential strategy for effectively treating Alzheimer disease.

Isoprenoids and prenylated proteins have been implicated in the pathophysiology of Alzheimer disease (AD), including amyloid-β precursor protein metabolism, Tau phosphorylation, synaptic plasticity, and neuroinflammation. However, little is known about the relative importance of the two protein prenyltransferases, farnesyltransferase (FT) and geranylgeranyltransferase-1 (GGT), in the pathogenesis of AD. In this study, we defined the impact of deleting one copy of FT or GGT on the development of amyloid-β (Aβ)-associated neuropathology and learning/memory impairments in APPPS1 double transgenic mice, a well established model of AD. Heterozygous deletion of FT reduced Aβ deposition and neuroinflammation and rescued spatial learning and memory function in APPPS1 mice. Heterozygous deletion of GGT reduced the levels of Aβ and neuroinflammation but had no impact on learning and memory. These results document that farnesylation and geranylgeranylation play differential roles in AD pathogenesis and suggest that specific inhibition of protein farnesylation might be a potential strategy for effectively treating AD.

Alzheimer disease (AD)² accounts for approximately two-thirds of all dementia cases and affects more than 35 million individuals worldwide (1). To date, there is no cure for this devastating neurodegenerative disorder. Emerging evidence indicates that a post-translational lipid modification of proteins, known as prenylation, may play an important role in the pathogenesis of AD (2–4).

Prenylation is the covalent attachment of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) to a cysteine residue of proteins harboring a carboxyl-terminal CAAX motif (where C is cysteine, AA are usually aliphatic amino acids, and X is a variable). The isoprenoids FPP and GGPP are cholesterol biosynthetic intermediates, and their levels in cells can be reduced by statin therapy. Prenylation of CAAX proteins is catalyzed by protein farnesyltransferase (FT) and protein geranylgeranyltransferase-1 (GGT), and sequences within the CAAX motif determine whether a protein is farnesylated or geranylgeranylated. The Rab family proteins are geranylgeranylated by an unrelated enzyme called protein geranylgeranyltransferase-2 (GGT2 or RabGGT) (5, 6). The hydrophobic prenyl group facilitates anchoring of proteins in cell membranes and mediates protein-protein interactions. A variety of important intracellular proteins, including heterotrimeric G protein subunits and nuclear lamins, are prenylated, but the largest, and most extensively studied group, is the small GTase superfamily of Ras, Rab, and Rho proteins (7). Prenylated proteins regulate a wide range of cellular functions and are implicated in the pathogenesis of cancer, cardio- and cerebrovascular disorders, bone diseases, and progeria (7). Prenylation and prenylated proteins have also been implicated in the pathogenesis of neurodegenerative diseases, including AD (2–4).

Indeed, the inhibition of isoprenoid production and protein prenylation influences multiple aspects of AD (2–4). For example, statin-induced depletion of isoprenoids leads to reduced levels of protein prenylation and promotes nonamyloidogenic processing of APP and reduces the production of Aβ (8–11). Moreover, some nonsteroidal anti-inflammatory drugs reduce the production of Aβ42 by inhibiting the prenylated protein Rho and Rho-kinase (ROCK) (12), but others found no involvement of ROCK in the Aβ42-lowering activity of nonsteroidal
anti-inflammatory drugs (13). Inhibition of Rac also regulates APP expression and processing (14, 15). Conversely, supplementation of FPP and GGPP stimulates the production of Aβ (11, 12, 16). Consistent with these findings, the levels of FPP and GGPP are elevated in the brains of patients with AD (17), suggesting that the levels of prenylated proteins could be increased in AD brains. In addition to APP/Aβ metabolism, isoprenoids and prenylated proteins are involved in other aspects of AD pathology. For instance, inhibiting the prenylation of Rho GTPases reduces Aβ-induced neuroinflammation (18, 19). Also, limiting the availability of isoprenoids for prenylation protects neurons from Aβ-induced apoptosis via the activation of pro-survival signaling pathways (20–22). Inhibiting RhoA prenylation reduces total and phosphorylated Tau levels (23).

We and others have shown that manipulating the levels of isoprenoids and protein prenylation modulates synaptic plasticity and cognitive function in animal models (24–28). Moreover, activation of the prenylated protein Rac1 has been shown to play an important role in the development of AD. However, some important issues remain to be addressed. First, little is known about the relative importance of farnesylation and geranylgeranylation in the development of AD. Second, the majority of previous studies was conducted in vitro. Appropriate animal models had not been available to study the role of protein prenylation in AD. It is not known whether findings from in vitro studies can be replicated in animal models.

To define the role of the two protein prenylation pathways in the pathogenesis of AD in vivo, we used a genetic approach to directly manipulate the levels of FT and GGT activities in the APPPS1 mouse model of AD. Our results demonstrate for the first time that haplodeficiency in farnesyltransferase but not geranylgeranyltransferase rescues cognitive function as well as attenuates Aβ-associated neuropathology in APPPS1 mice.

**EXPERIMENTAL PROCEDURES**

**Animals—**APPPS1 double transgenic mice (B6C3-Tg [APPswe, PSEN1dE9] 85Db/Bo; stock number 004462) were purchased from The Jackson Laboratory (Bar Harbor, ME). FT or GGT β subunits heterozygous deletion (+/−) mice were generated as described previously (31). All transgenes are in the heterozygous state; therefore, breeding of APPPS1 mice with either FT+/− or GGT+/− mice produced four genotypes of mice, respectively, APPPS1/FT+/−, APPPS1/FT−/−, APPPS1/GGT+/−, APPPS1/GGT−/−, and wild-type (WT) mice or APPPS1/GGT+/−, APPPS1/GGT−/−, and WT mice. Genotypes of the mice were determined by PCR analysis of genomic DNA from tail biopsies with gene-specific primers. All animal procedures used for this study were prospectively reviewed and approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

**Blood Collection and Brain Tissue Preparation—**The mice were deeply anesthetized, and blood was collected by cardiac puncture with heparin as an anticoagulant. Following perfusion via the heart with ice-cold PBS, brains were cut sagittally into left and right hemispheres. The left hemisphere was fixed in 4% paraformalde-hyde for histological analysis. The right hemisphere (devoid of cer-ebellum and brain stem) was snap-frozen in liquid nitrogen and stored at −80°C for biochemical analysis.

**Brain Aβ ELISA and Immunoblot Analysis—**Brain homogenates were prepared as we described previously (32, 33). Commercial ELISA kits (Invitrogen) were used to measure Aβ40 and Aβ42 levels in carbonate-soluble and -insoluble (guanidine-soluble) fractions according to the manufacturer’s protocol. For immunoblot analysis, aliquots of brain homogenate were separated by SDS-PAGE and blotted to nitrocellulose membranes. The membranes were incubated with specific primary antibodies against the following: human Aβ (6E10, Signet, Dedham, MA); the carboxyl terminus of APP (CT695, Invitrogen); the soluble amino-terminal fragments of APP (sAPPα and sAPPβ) (2B3 (11088B) and 6A1 (10321B), Clontech), GGT-1β (sc-18996), and FT-β (sc-137) (Santa Cruz Biotechnology, Dallas, TX); mouse apolipoprotein E (apoE) (sc-6384, Santa Cruz Biotechnology); low density lipoprotein receptor-related protein 1 (LRP1) (kindly provided by Dr. Guojun Bu, Mayo Clinic, Jacksonville, FL); insulin-degrading enzyme (IDE) (PC730, EMD Biosciences/Millipore, Billerica, MA); neprilysin (sc-9149, Santa Cruz Biotechnology); IBA-1 (ionized calcium-binding adaptor molecule 1) (Wako, Richmond, VA), and GFAP (glial fibrillary acidic protein) (Millipore) followed by HRP-conjugated secondary antibodies. Signal was detected by the ECL Plus Western Blotting System (GE Healthcare) and quantified by the ImageJ software. For a loading control when appropriate, the blots were stripped and reprobed with mouse anti-actin or anti-tubulin monoclonal antibody (Sigma) or goat anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal antibody (sc-20357, Santa Cruz Biotechnology).

**Immunohistochemical Analysis and Quantification of Aβ Deposition, Activated Astrocytes, and Activated Microglia—**Protocols for immunohistochemical analysis have been described previously (32–34). Briefly, fixed brain tissues were sectioned at 50 μm using a Vibratome (Leica Microsystems Inc). Tissue sections were stored at 4°C in PBS with 0.01% sodium azide and subjected to free-floating immunostaining using the ABC kit (Vector Laboratories, Burlingame, CA) to detect Aβ, activated microglia, and astrocytes. The primary antibody 6E10 (Signet) was used for assessing Aβ deposition, IBA-1 antibody (Wako) for assessing activated microglia, and GFAP antibody (Millipore) for IBA-1 and GFAP, and IBA-1 in the cortex and hippocampus of mouse brain was quantified using a histomorphometry system (Image-Pro Plus, MediaCybernetics, Rockville, MD).

For double immunofluorescence analyses, sections were incubated overnight at 4°C in a mixture of IBA-1 or GFAP antibody (rabbit) with 6E10 antibody (mouse). After the incubation, a combination of secondary antibodies, Alexa 488-tagged donkey anti-rabbit IgG and Alexa 594-tagged donkey anti-mouse IgG (Molecular Probe, Eugene, OR), were applied for 60 min at room temperature. The sections were examined with an Olympus FluoView FV1000 BX2 upright confocal microscopic system.

**Behavioral Assessment—**Three AD-related behavioral functions (spatial learning and memory, exploration of environmental stimuli, and anxiety) were assessed. The testing schedule included exploration of the T-maze (days 1–5), the open
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RESULTS

Haplodeficiency of FT or GGT in APPPS1 Mice—Breeding of APPPS1 mice with FT+/− or GGT+/− mice produced mice that carry the APPPS1 double transgenes and heterozygous deletion of the FT or GGT β subunit. To verify the haplodeficiency of FT and GGT in the brain, we assessed protein levels of FT and GGT in lysates of the cortex. As expected, Western blot analysis indicated that the protein level of FT and GGT was decreased by ~50% in APPPS1/FT+/− and APPPS1/GGT+/− mice, respectively, compared with that in APPPS1 mice (Fig. 1).

Reduced Steady-state Levels and Deposition of Aβ in Brains of APPPS1/FT+/− and APPPS1/GGT+/− Mice—To define the impact of FT and GGT haplodeficiency on cerebral β-amyloidogenesis in APPPS1 mice, we quantified the levels of Aβ in the carbonate-soluble and -insoluble (guanidine-soluble) fractions in brain lysates by ELISA. In the carbonate-soluble fraction, there was no difference in Aβ42 levels between the different groups, but the level of soluble Aβ40 was decreased in APPPS1/GGT+/− mice (Fig. 2A). In the guanidine-soluble fraction, the levels of Aβ40 and Aβ42 were reduced in both APPPS1/FT+/− and APPPS1/GGT+/− mice, compared with

Statistical Analysis—Data are expressed as means ± S.E. Comparison of different genotype groups was performed by Student’s t test and repeated measures of analysis of variance. *p < 0.05; **p < 0.01.

field (days 1–3), the elevated plus-maze test for anxiety levels (days 4 and 5), and spatial learning in the Morris water maze (days 6–11). All equipment and software were purchased from SD Instruments (San Diego). All testing procedures have been described in detail previously (26, 32, 33).

Briefly, the Morris water maze consists of a round basin filled with water. The room contained abundant extra-maze visual cues for orientation. The acquisition of the spatial task consists of placing the mice next to and facing the wall successively in north, east, south, and west positions, with the escape platform hidden 1 cm below the water level in the middle of the northeast quadrant. In each trial, the mouse was allowed to swim until it finds the hidden platform or until 60 s had elapsed, at which point the mouse was guided to the platform, where it remained for 10 s before being returned to a cage containing paper towels to dry. The escape latency and swim path length were recorded by the ANYMAZE System (San Diego Instruments, San Diego). All testing procedures have been described in detail previously (26, 32, 33).

Comparison of different genotype groups was performed by Student’s t test and repeated measures of analysis of variance. *p < 0.05; **p < 0.01.

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APPPS1 (Fig. 2B). Consistent with these findings, Aβ deposition in the hippocampal area was lower in APPPS1/FT+/− and APPPS1/GGT+/− mice than in APPPS1 mice (Fig. 3, A and B). However, the reduction was more pronounced in APPPS1/FT+/− mice than in APPPS1/GGT+/− mice (Fig. 3B). Aβ deposition in the cortex area was reduced in APPPS1/FT+/− compared with APPPS1 mice, but there was only a trend for a decrease in APPPS1/GGT+/− mice (Fig. 3, C and D).

**FT Haplodeficiency Enhances Nonamyloidogenic Processing of APP and Degradation of Aβ**—To investigate whether the decrease of Aβ levels in the brain of APPPS1/FT+/− and APPPS1/GGT+/− mice was caused by a decrease in the generation of Aβ from APP, we determined the steady-state levels of full-length APP, and carboxyl-terminal fragments (CTF) and soluble amino-terminal fragments (sAPP) of APP produced by α- and β-secretase cleavages by immunoblot analyses. There were no differences in the amount of full-length APP, but the ratios of β-CTF to α-CTF and sAPPβ to sAPPα in APPPS1/FT+/− mice were reduced (Fig. 4, A and B), suggesting a shift to nonamyloidogenic processing of APP by FT haplodeficiency.

Next, we examined factors that are involved in Aβ clearance and degradation. ApoE and LRP1 are major proteins involved in the clearance of Aβ in the brain (35). Immunoblot analysis indicated that the steady-state levels of apoE and LRP1 were unchanged. IDE, originally recognized for its ability to degrade insulin, can also degrade Aβ (36). Interestingly, inhibition of isoprenoid biosynthesis has been found to stimulate IDE secretion (37). The steady-state levels of IDE were higher in APPPS1/FT+/− than in APPPS1/GGT+/− mice (Fig. 2).

**FIGURE 3. Aβ deposition is reduced to different degrees in the hippocampal and cortical areas of FT- and GGT-haplodeficient mice.** A and C, representative brain sections of hippocampal (A) and cortical (C) areas from 6- and 9-month-old mice immunostained with anti-Aβ antibody (6E10). B and D, relative Aβ load in the hippocampal (B) and cortical (D) areas determined by immunohistochemical and morphometric analyses with the levels in the age- and sex-matched APPPS1 control group set as 100%. In the hippocampal area, Aβ deposition is reduced in both APPPS1/FT+/− and APPPS1/GGT+/− mice. In the cortical area, the Aβ deposition is reduced significantly in APPPS1/FT+/− mice but not in APPPS1/GGT+/− mice. n = 7–10 mice/genotype; age = 6–9 months; *, p < 0.05; **, p < 0.01. Scale bars, 100 μm.

**FIGURE 4. Effect of FT and GGT haplodeficiency on APP processing.** A, immunoblot analysis of full-length APP (FL-APP), α- and β-CTF of APP, and sAPPα and sAPPβ of APP. B, densitometric analysis of immunoblots (normalized by the amount of tubulin) with the levels in the control group set as 100%. The ratios of β-CTF to α-CTF (CTF−β/α) and sAPPβ to sAPPα (sAPP-β/α) are significantly decreased in APPPS1/FT+/− mice. n = 7–10 mice/genotype; age = 6–9 months; *, p < 0.05.
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FT+/− than in APPPS1 mice (Fig. 5, A and B); the levels of IDE in APPPS1/GGT+/− mice were unchanged. We also analyzed another Aβ-degrading enzyme, nephrilysin (NEP), and we found no change in the level of NEP between different genotypes of mice. These findings suggest that FT haplodeficiency enhances Aβ degradation via up-regulation of IDE.

Reduced Neuroinflammation in APPPS1/FT+/− and APPPS1/GGT+/− Mice—Activation of microglia and astrocytes and other inflammatory responses are common features in brains of AD patients. To identify activated microglia, mouse brain sections were stained with an antibody against IBA-1, a marker of microgliosis (Fig. 6A). IBA-1 staining was reduced in APPPS1/FT+/− and APPPS1/GGT+/− brains compared with APPPS1 brains (Fig. 6B). We also analyzed the extent of astrocyte activation (GFAP) by immunohistochemical analyses. Astrocyte activation was also reduced in APPPS1/FT+/− and APPPS1/GGT+/− brains compared with APPPS1 brains (Fig. 6, A and B). Western blot analyses of IBA-1 and GFAP in brain homogenates showed similar results as the immunohistochemical analyses (Fig. 6, C and D). These findings demonstrate that FT or GGT haplodeficiency attenuates neuroinflammation marked by microglial and astrocyte activation.

FT Haplodeficiency but Not GGT Haplodeficiency Rescues Learning and Memory Function in APPPS1 Mice—We performed a battery of behavioral tests to determine whether heterozygous deletion of FT and GGT affects learning and memory functions in APPPS1 mice. The Morris water maze test was performed to assess the spatial learning and memory of APPPS1/FT+/− and APPPS1/GGT+/− mice (9–11 months of age). These findings demonstrate that FT or GGT haplodeficiency attenuates neuroinflammation marked by microglial and astrocyte activation.

FIGURE 5. Effect of FT and GGT haplodeficiency on proteins involved in clearance/degradation of Aβ. A, immunoblot analysis of ApoE, LRP1, IDE, and NEP levels in the cerebral homogenates. B, densitometric analysis of immunoblots (normalized by the amount of β-actin), with the levels in the control group set as 100%. The level of IDE is significantly increased in APPPS1/FT+/− mice. n = 7–10 mice/genotype; age = 6–9 months; *, p < 0.05.

FIGURE 6. Attenuated neuroinflammation in the brain of FT- and GGT-haplodeficient APPPS1 mice. A, representative brain sections from APPPS1, APPPS1/FT+/−, and APPPS1/GGT+/− mice immunostained with an antibody against activated microglial marker IBA-1 (panels a–f) and activated astrocyte marker GFAP (panels g–o). Confocal microscopic images showing colocalization of Aβ (red) and IBA-1 (green) (panels g–i) or GFAP (green) (panels p–r). B, relative percentage area covered by IBA-1 or GFAP immunostaining in the cortex with the level in the age- and sex-matched APPPS1 control group set as 100%. C, immunoblot analysis of IBA-1 and GFAP level in the cerebral homogenates. D, densitometric analysis of IBA-1 and GFAP immunoblots (normalized by the amount of β-actin), with the levels in the control group set as 100%. Scale bars, 100 μm in panels a–c, j, and k; 50 μm in panels d–f and m–o; 10 μm in panels g–i and p–r.
(Fig. 7A). Interestingly, APPPS1/FT\(^{+/−}\) mice performed similarly as WT mice, whereas APPPS1/GGT\(^{+/−}\) mice performed similarly as APPPS1 mice (Fig. 7A).

To assess memory retention following acquisition, the mice were subjected to a single probe trial 24 h later. As expected, WT mice spent 42.9 ± 3.7% of the time in the target quadrant, whereas APPPS1 mice spent only 30.7 ± 2.8% of the time there (not different from the random 25%), demonstrating memory deficits in APPPS1 mice (Fig. 7B). Consistent with findings in the acquisition phase, APPPS1/FT\(^{+/−}\) performed similarly as WT mice and spent significantly more time (49.4 ± 3.8%) in the target quadrant than APPPS1 mice (Fig. 7B). In contrast, APPPS1/GGT\(^{+/−}\) mice performed as poorly as APPPS1 mice and spent only 34.7 ± 4.3% of the time in the target quadrant (Fig. 7B). Thus, reduced expression of FT (but not GGT) rescues APPPS1 mice from the development of learning and memory deficits.

In the visible test of the Morris water maze, all groups performed similarly (escape latency = 8–11 s), indicating no changes in visual acuities or swimming speeds. In addition, neither FT nor GGT haplo deficiency had any significant effects on motor activity in an open field test, exploration in a T-maze test, or on anxiety in an elevated plus-maze test (Table 1). These data suggest that the superior learning and memory performance of APPPS1/FT\(^{+/−}\) mice was not caused by other noncognitive behavioral changes.

### DISCUSSION

The majority of previous studies investigating the effects of isoprenoids and/or protein prenylation on AD-related processes/functions were conducted in vitro, and statins were often used as a pharmacological tool (for recent reviews, see Refs. 2–4). Although statins can effectively manipulate the levels of isoprenoids (17), there are some caveats in this approach. First, statins reduce the level of the isoprenoid substrates for protein prenylation but not the level of protein prenylation per se. Depending on the dynamics of protein prenylation and the turnover rate of prenylated proteins, the levels of isoprenoids may or may not affect the level of prenylated proteins. Second, statins decrease the levels of FPP and GGPP simultaneously (17) and thus may affect both farnesylation and geranylgeranylation pathways. Importantly, farnesylated and geranylgeranylated proteins are involved in regulating distinct cellular functions (38). Thus, it is difficult to dissect the roles of the two prenylation pathways using statins. Also, depending on the type, dose, and treatment duration of statins, the two prenylation pathways may be affected to different degrees (39), which may lead to inconsistent outcomes. Third, isoprenoids have been shown to possess prenylation-independent effects on APP processing (16) and expression of small GTPases (40). Thus statin-induced isoprenoid effects may not result from changes in protein prenylation. Finally, isoprenoid-independent effects

### TABLE 1

**Exploratory activities and anxiety levels of APPPS1 mice**  
\(n = 7–14\) mice/genotype.

| Tests                                 | WT          | APPPS1     | APPPS1/FT\(^{+/−}\) | APPPS1/GGT\(^{+/−}\) |
|---------------------------------------|-------------|------------|----------------------|-----------------------|
| **Spontaneous alternation (T-maze)**  |             |            |                      |                       |
| Rate                                  | 71.0 ± 5.0% | 61.0 ± 5.0%| 64.0 ± 5.0%          | 63.0 ± 6.0%           |
| Latency                               | 18.6 ± 3.4 s| 29.4 ± 4.2 s| 18.0 ± 4.3 s         | 18.0 ± 5.5 s          |
| **Motor activity (open field)**       |             |            |                      |                       |
| Path length (cm)                      |             |            |                      |                       |
| Day 1                                 | 2782.6 ± 410.2 | 2021.3 ± 465.2 | 2553.6 ± 746.8 | 3561.1 ± 768.9 |
| Day 2                                 | 1899.2 ± 414.5 | 1476.4 ± 293.6 | 2687.0 ± 598.4 | 2416.0 ± 599.0 |
| Day 3                                 | 1922.1 ± 391.6 | 1519.1 ± 301.3 | 2346.4 ± 501.3 | 2035.4 ± 454.1 |
| Percentage of time in the central zone|             |            |                      |                       |
| Day 1                                 | 1.6 ± 0.5   | 0.9 ± 0.4  | 1.8 ± 0.5            | 1.4 ± 0.4             |
| Day 2                                 | 1.2 ± 0.7   | 0.6 ± 0.3  | 1.4 ± 0.4            | 0.7 ± 0.3             |
| Day 3                                 | 0.5 ± 0.2   | 0.6 ± 0.4  | 0.7 ± 0.3            | 0.4 ± 0.3             |
| **Anxiety (elevated plus-maze)**      |             |            |                      |                       |
| Entries to open arms                  |             |            |                      |                       |
| Day 1                                 | 7.0 ± 1.7   | 6.5 ± 1.6  | 9.0 ± 1.5            | 8.6 ± 1.9             |
| Day 2                                 | 2.4 ± 0.7   | 1.8 ± 0.5  | 3.8 ± 1.4            | 1.6 ± 0.7             |
| Percentage of time in open arms       |             |            |                      |                       |
| Day 1                                 | 17.2 ± 6.0  | 18.9 ± 5.9 | 25.3 ± 7.7           | 14.9 ± 4.9            |
| Day 2                                 | 4.5 ± 1.5   | 4.1 ± 1.5  | 10.4 ± 3.3           | 2.2 ± 1.2             |
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of statins, such as cholesterol-lowering effects, further complicate the interpretation of experimental results. To overcome some of the limitations of previous studies, we took a genetic approach and deleted one copy of FT and GGT in APPPS1 mice. Our results demonstrate for the first time that either FT or GGT haploinsufficiency attenuates Aβ-associated neuropathology but only FT haploinsufficiency rescues cognitive function in the APPPS1 mouse model of AD.

Both farnesylated and geranylgeranylated proteins have been implicated in regulating the processing of APP and the production/secretion of Aβ (2–4). For example, geranylgeranylated RhoA-mediated activation of ROCK increases Aβ42 secretion via modulation of γ-secretase (12), whereas specific inhibition of the farnesylated Rho/ROCK pathway promotes α-secretase activity (8). Interestingly, although inhibitors of ROCK reduce total Aβ secretion, targeting ROCK by expression of dominant-negative or constitutively active ROCK mutants failed to modulate Aβ secretion (13). Additional in vitro studies show that statin-induced low isoprenoid conditions cause the accumulation of intracellular APP, β-CTF, and Aβ, which can be rescued by GGPP supplementation, suggesting the involvement of geranylgeranylated target proteins (9). The study also shows that low isoprenoid levels inhibit the trafficking of APP through the secretory pathway (9). A more recent study further demonstrates that low isoprenoid conditions induced by physiologically relevant doses of statins preferentially inhibit the geranylgeranylation of Rab family proteins involved in vesicle trafficking and thereby affect the trafficking and intracellular localization of APP (10). Because the majority of Rab proteins require two GGPP molecules to be geranylgeranylated (6), the function of this family of proteins would be compromised first under the condition of limited GGPP. It is also worth noting that the statin-induced low level of isoprenoids usually reduces protein geranylgeranylation readily, whereas protein farnesylation is preserved due to a particularly strong affinity of FT for FPP (39). This may explain why in some studies, supplementation of FPP had no effects on statin-induced changes, which may have potentially led to the dismissal of the involvement of farnesylated proteins in relevant pathways when statins were used as a pharmacological tool (9, 10). In this study, RabGGT activity and the isoprenoid production pathway were intact. Without the potential complications of intracellular trafficking and substrate availability, this study shows that either FT or GGT haploinsufficiency reduces the levels of Aβ40 and Aβ42 in APPPS1 mice. The mechanisms for the reduced Aβ levels are not fully understood. In FT+/− mice, a decrease in CTFβ/α and sAPPβ/α ratios suggests a stimulation of the nonamyloidogenic α-secretase processing of APP by FT haploinsufficiency, which is consistent with the findings that pharmacological inhibition of farnesylation with an FT inhibitor (FTI) promotes α-secretase activity in neuronal cells (8). Additionally, treatment with an FTI efficiently stimulated the secretion of IDE by microglia, whereas the geranylgeranyltransferase inhibitor (GGTI) had no effect (37). Furthermore, this FTI-induced IDE secretion led to a concomitant increase in the degradation of Aβ in the conditioned media of microglia (37). Consistent with these findings, the level of IDE is upregulated in the brain of APPPS1/FT+/− mice. Thus, FT may affect the level of Aβ partly by modulating APP processing and Aβ degradation. However, it is less clear on the mechanisms by which GGT haploinsufficiency reduces the level of Aβ. No change was observed in the ratios of CTFβ/α and sAPPβ/α in APPPS1/GGT+/− mice. Previous studies in vitro indicate that different members in the Rho small GTPase family are involved in modulating the activities of α-, β-, and γ-secretases (2–4). In GGT haploinsufficient mice, multiple Rho GTPases that depend on geranylgeranylation for function could be affected. Therefore, it is possible that the activities of all three secretases are altered, leading to no net change in the ratios of CTFβ/α and sAPPβ/α. A clearer understanding on the role of GGT in APP/Aβ metabolism requires further investigation.

The role of protein prenylation in neuroinflammation had also been explored in vitro, but the results were contradictory. Using statin-treated hippocampal slice preparations, one study showed that inhibition of protein geranylgeranylation elicited microglial activation (41). Conversely, another study demonstrated that statin-induced inhibition of protein geranylgeranylation suppressed the Aβ-stimulated inflammation in microglial cultures (18). The discrepancies between these studies may result from specific experimental conditions in vitro and/or complications of statin actions. In this study, we show that either FT or GGT haploinsufficiency attenuated Aβ-associated neuroinflammation in APPPS1 mice, suggesting the involvement of both farnesylated and geranylgeranylated proteins in regulating neuroinflammation and the potential use of FTIs or GGTIs as anti-inflammatory agents. However, caution must be taken when considering GGTIs as therapeutic agents as a recent study reported erosive inflammatory arthritis in mice with the deletion of GGT in macrophages (42).

The most striking observation of this study is that FT haploinsufficiency but not GGT haploinsufficiency rescued learning and memory function in APPPS1 mice as well as attenuated Aβ pathology and neuroinflammation. Although the exact underlying mechanisms require further investigation, this differential effect of FT and GGT on cognitive function may reflect distinct functions of farnesylated and geranylgeranylated proteins in memory pathways. The small GTPases, which include the two major families Ras and Rho, are differentially regulated by farnesylation and geranylgeranylation, respectively. These proteins play important roles in signaling pathways for neuronal plasticity and memory formation (27). Several lines of evidence indicate that H-Ras, an exclusively farnesylated protein, negatively regulates synaptic plasticity and spatial learning/memory. Specifically, it has been shown that H-Ras overexpression decreases synaptic plasticity in hippocampal neurons (43). Consistently, neurotransmitter receptors are activated, and synaptic plasticity is augmented in H-Ras-deficient mice (44). In a recent study, we demonstrated that inhibiting the level of FPP and farnesylation but not GGPP and geranylgeranylation enhances synaptic plasticity in hippocampal slices (25). In addition, in a mouse model of neurofibromatosis type 1 mental retardation, in which excessive Ras activity impairs synaptic function (45), inhibition of farnesylation of Ras reverses synaptic and spatial learning deficits (45). These findings strongly suggest that inhibition of farnesylated proteins such as Ras can augment neuroplasticity/spatial learning and may partly...
explain why in the current investigation FT haplodeficiency rescued cognitive function in APPPS1 mice. However, geranylgeranylated Rho family proteins are crucial in synapse/spine formation and remodeling (46). Studies have shown that fully active GGT not only is required for the activation of Rho GTases but also could be a signaling molecule itself (47). In addition, more proteins are subjected to geranylgeranylation than farnesylation (48). It is possible that some geranylgeranylation-dependent functions could be compromised in GGT−/− mice. Therefore, the detrimental effects of GGT haplodeficiency might have neutralized the benefits conferred by the attenuation of AD-related neuropathology in APPPS1/GGT−/− mice.

In summary, our results demonstrate that heterozygous deletion of FT and GGT differentially affects the course of AD. Although either FT or GGT haplodeficiency reduces Aβ deposition and neuroinflammation, only FT haplodeficiency rescues cognitive function in APPPS1 mice. Thus, pharmacological agents that inhibit both prenylation pathways, such as statins, may produce confounding results, which may partly explain some inconsistent outcomes in statutes clinical trials in AD patients. Our findings strongly suggest that specific inhibition of FT activity may present a novel and effective strategy for the prevention and/or treatment of AD.

Acknowledgments—We thank Andrea Gram, Fuyuan Wang, and Laurie Baeker Hovde for technical and administrative assistance. We thank Drs. Gibson Wood, Gunter Eckert, and Mark Distefano for helpful discussions on the data. We also thank Drs. Guojun Bu and Takahisa Kanekiyo for providing the antibody against LRPI and the instructions for using the antibody.

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