HHS Public Access

Author manuscript

J Alzheimers Dis. Author manuscript; available in PMC 2024 January 01.

Published in final edited form as:

J Alzheimers Dis. 2023 ; 95(2): 399–405. doi:10.3233/JAD-230218.

Upregulation of sFRP1 is more profound in female than male 5xFAD mice and positively associated with amyloid pathology

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Abstract

The prevalence of Alzheimer's disease is greater in women, but the underlying mechanisms remain to be elucidated. We herein demonstrated that α-secretase ADAM10 was downregulated and ADAM10 inhibitor sFRP1 was upregulated in 5xFAD mice. While there were no sex effects on ADAM10 protein and sFRP1 mRNA levels, female 5xFAD and age-matched non-transgenic mice exhibited higher levels of sFRP1 protein than corresponding male mice. Importantly, female 5xFAD mice accumulated more Aβ than males, and sFRP1 protein levels were positively associated with Aβ42 levels in 5xFAD mice. Our study suggests that sFRP1 is associated with amyloid pathology in a sex-dependent manner.

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Authors' contributions

Y.L. designed the experiments. G.B., T.K., and C-C. Liu contributed to scientific discussions. Y. M. provided technical support on Aβ40/42 ELISA assays. J.R.M., N.W., W.L., S.J., F.S., and Y.L. conducted experiments and/or analyzed data. The paper was drafted by Y.L., and all authors read, edited, and approved the manuscript.

Declarations

Ethics Approval and Consent to Participate

All animal experiments were conducted in accordance with NIH guidelines for the care and use of laboratory animals and were reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC).

Consent for Publication

All authors gave their consent for publication.

Conflict of Interest

G.B. is currently an employee of SciNeuro pharmaceuticals. The other authors declare no competing interests.

Keywords

sFRP1; amyloid β (Aβ); AβPP; sex-dependent effects; 5xFAD mice

Introduction

Alzheimer's disease (AD) is the most common form of age-related dementia with the pathological amyloid-β (Aβ) plaques and tau-containing neurofibrillary tangles as the two major hallmarks of this incurable disease. Amyloid plaques are comprised of amyloid-β (Aβ) peptides that accumulate in the extracellular space between neurons [1, 2]. Aβ is one of the downstream products of Amyloid-β Precursor Protein (AβPP) processing, generated when AβPP is sequentially cleaved by β-secretase and γ-secretase. However, AβPP can also be cleaved by the non-amyloidogenic pathway via α-secretase to generate non-toxic fragments [3]. Since α-secretase cleaves AβPP within the Aβ sequence, it prevents the Aβ formation. ADAM10, a member of the A Disintegrin And Metalloproteinase (ADAM) family, is a physiologically relevant α-secretase of AβPP in neurons [4, 5]. Genetic and functional studies demonstrate that the diminished α-secretase activity via ADAM10 missense mutations leads to increased Aβ pathology in AD [6, 7]. Further, neuronal overexpression of ADAM10 enhances AβPP cleavage via the α-secretase pathway, resulting in reduced amyloid plaque formation and attenuated cognitive decline in mice transgenic for human $AβPP_{([V717I])}$ [8]. Therefore, the upregulation of ADAM10 activity is an attractive therapeutic strategy for AD [9–11].

Secreted frizzled-related proteins (sFRPs) have been characterized as Wnt antagonists, owing to similarities in their structure with the extracellular domain of frizzled proteins [12]. Interestingly, these proteins, sFRP1 in particular, have been recently shown to bind to ADAM10 and consequently inhibit its activity [13]. Notedly, the sFRP1 protein level is upregulated in the brain of AD patients and positively correlates with amyloidogenic AβPP processing [14–16]. By performing deep multilayer human brain proteomics, Bai *et al* identified sFRP1 as one of the fifteen Aβ-correlated proteins in human AD brains, which is also validated by the Alzheimer mouse model 5xFAD [16]. While sFRP1 overexpression in the Alzheimer APP/PS1 mouse model accelerates the development of amyloid plaques and dystrophic neurites, its genetic inactivation or antibody-mediated neutralization favors non-amyloidogenic AβPP processing, suggesting that sFRP1 is an important player in AD pathogenesis [14].

There are sex differences in the AD clinical phenotype and progression, and AD is more prevalent in elderly women, accompanied with more severe Aβ-associated pathology [17–19]. The Alzheimer mouse model 5xFAD expresses human AβPP and presenilin 1 with five familial AD mutations and recapitulates many AD-related phenotypes [20]. 5xFAD mice start to develop visible amyloid deposition as early as 2 months of age, and display deficits in spatial learning at 4–5 months of age [20]. Interestingly, female 5xFAD mice exhibit more profound amyloid pathology than 5xFAD males [20–22]. Therefore, in the present study, we aimed to determine whether there is a sex-dependent effect on sFRP1 upregulation in 5xFAD mice and if so, whether the sFRP1 level is associated with amyloid pathology.

Materials and methods

5xFAD mice

5xFAD mice were obtained from Jackson Labs and were maintained in a heterozygote state in a C57BL/6 background. All experiments were performed in accordance with NIH guidelines for the care and use of laboratory animals and were reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC). 5xFAD mice (sex mixed) at 14 months of age were sacrificed, and cortex from left hemisphere was snap-frozen in liquid nitrogen immediately and stored at −80°C for further studies.

Quantitative Real-Time PCR of sFRP1

Mouse cortex samples were homogenized in Trizol (Life Technologies, Carlsbad, CA, USA) and RNA was extracted as previously described [23]. Mouse sFRP1 real-time primer set (PPM04438C-200) and mouse GAPDH real-time primer set (PPM02946E-200) were purchased from Qiagen.

Preparation of mouse brain TBS-T and FA fractions

Mouse cortex samples of 5xFAD mice and age-matched non-transgenic (NTG) littermates were lysed with Tris-buffered saline containing 1% Triton X-100 (TBS-T), protease inhibitor (Roche Diagnostics GmbH, Mannheim, Germany) and PHOSSTOP phosphatase inhibitor (Roche Diagnostics GmbH, Mannheim, Germany). The TBS-T insoluble pellets were re-suspended in 70% formic acid (FA). The preparation of the TBS-T and FA soluble fractions were described before [23, 24].

Western blotting

Western blot analyses were conducted under reducing conditions as described before [23]. Specially, 16 μg protein of each TBS-T fraction and 1.2 μg protein of each FA fraction were loaded per well. The primary antibodies and their dilutions used in this study are as follows: anti-sFRP1 (Invitrogen, MA5–38193, 1:1000), anti-ADAM10 (Santa Cruz Biotechnology, SC-28358, 1:1000) and anti-β-actin (Sigma Life Sciences, A2228, 1:5000). The membrane for detecting sFRP1 in FA fraction was restained with Revert 700 total protein stain solution (LI-COR Biosciences) for total protein quantification.

Measurement of Aβ40 and Aβ42 levels

Enzyme linked immunosorbent assay (ELISA) was used to measure the levels of Aβ40 and Aβ42 in TBS-T and FR fractions from the cortex of 5xFAD mice as described before [24]. Briefly, Aβ40 was captured with 13.1.1 monoclonal antibody (anti-Aβ 35–40) and Aβ42 was captured with 2.1.3 monoclonal antibody (anti-Aβ 35–42). Aβ was then detected with HRP conjugated Ab5 monoclonal antibody. HRP activity was assayed with 3,3’,5,5’-Tetramethylbenzidine (TMB) Super Slow (Sigma-Aldrich, St. Louis, MO, USA). The levels of Aβ40 and Aβ42 in TBS-T and FA fractions were normalized with protein concentrations.

Statistical analyses

Statistical analyses were performed with the GraphPad Prism 9 software unless noted otherwise, and all data were presented as mean values ± SEM. Unpaired t test was performed for comparison of two groups, and two-way ANOVA was used with Tukey's multiple comparison test as a post-hoc test. *, P < 0.5; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

Results

Downregulation of ADAM10 and upregulation of sFRP1 in 5xFAD mice

5xFAD mice recapitulate many AD-related phenotypes including an age-dependent extracellular plaque deposition and substantial neuron loss in cortical layer 5 at 12 months of age [25]. To characterize AβPP processing towards the nonpathogenic pathway, we studied ADAM and sFRP1 levels in the cortex of 5xFAD mice at 14 months of age. Compared to age-matched NTG control mice, 5xFAD mice displayed a 22 ± 4 % decrease of ADAM10 protein level ($F_{(1,20)} = 21.64$, $p < 0.001$) (Fig. 1A & 1B). In addition, there was a 43 ± 7% increase of sFRP1 mRNA level in 5xFAD mice ($F_{(1,26)} = 25.35$, $p < 0.0001$) (Fig. 1C). Interestingly, the sFRP1 protein levels in the TBS-T fractions of the cortex of male and female 5xFAD mice were 4.3 ± 0.5-folds ($p < 0.0001$) and 4.1 ± 0.6-folds ($P < 0.001$) of corresponding NTG male and female mice, respectively (Fig. 1D & 1E). Formic acid (FA) is generally applied to solubilize Aβ from fibril plaques [26]. sFRP1 was found in the FA fractions of the cortex of male and female 5xFAD mice but was undetectable in the FA fractions of the cortex of male and female NTG mice (data not shown). Taken together, these data suggest that both ADAM10 downregulation and sFRP1 upregulation could contribute to inhibition of AβPP processing towards the nonpathogenic pathway, and that abnormal secretion of sFRP1 occurs not only at the transcriptional level but also through posttranscriptional mechanisms.

Upregulation of sFRP1 is sex-dependent in 5xFAD mice

While there were no sex effects on ADAM protein and sFRP1 mRNA levels in 5xFAD mice and NTG mice (Fig. 1A–1C), the sFRP1 protein levels in the TBS-T fractions from the cortex of female 5xFAD mice and female NTG mice were 3.2 ± 0.3-folds ($p < 0.0001$) and 4.2 ± 0.3-folds ($p < 0.0001$) of corresponding male 5xFAD mice and male NTG mice, respectively (Fig. 2A & 2B). Although sFRP1 was undetectable in the FA fractions of the cortex of both male and female NTG mice, sFRP1 protein levels in the FA fractions from the cortex of female 5xFAD mice were 2.7 ± 0.5-folds ($p < 0.05$) of male 5xFAD mice (Fig.2C & 2D). Together, our results indicate that there is a sex-dependent effect on sFRP1 upregulation mediated by posttranscriptional mechanisms.

5xFAD female mice exhibits higher levels of Aβ40 and Aβ42 than 5xFAD male mice

Female 5xFAD mice accumulate more Aβ than males [20–22]. We also found that the levels of Aβ40 and Aβ42 in the TBS-T fractions from the cortex of female 5xFAD mice were higher than those of male 5xFAD mice (Aβ40 at 1.10 ± 0.16 pg/μg protein for female 5xFAD mice vs 0.58 ± 0.08 pg/μg protein for male mice, $P < 0.05$; Aβ42 at 8.41 ± 1.27

pg/μg protein for female 5xFAD mice vs 3.16 ± 0.68 pg/μg protein for male mic, $P < 0.01$) (Fig. 3A & 3B). In addition, the levels of Aβ40 and Aβ42 in the FA fractions from the cortex of female 5xFAD mice were higher than those of male 5xFAD mice (Aβ40 at 1008 ± 84 pg/μg protein for female 5xFAD mice vs 580 ± 112 pg/μg protein for male mice, $P < 0.05$; Aβ42 at 1219 ± 152 pg/μg protein for female 5xFAD mice vs 999 ± 158 pg/μg protein for male mic, $P > 0.05$) (Fig. 3C & 3D). However, the Aβ42/Aβ40 ratio was insignificantly changes between male and female 5xFAD mice (Fig. 3E & 3F).

3.4. Upregulation of sFRP1 is associated with amyloid pathology in 5xFAD mice

Having established that there are sex-dependent effects on sFRP1 and Aβ levels in 5xFAD mice, we then determined the correlation between sFRP1 and Aβ levels. Although sFRP1 protein levels in the FA fractions were not associated with Aβ40 and Aβ42 levels in the FA fractions from the cortex of 5xFAD mice (Fig. 3I & 3J), sFRP1 protein levels in the TBS-T fractions were positively corelated with Aβ42 levels in the TBS-T fractions from the cortex of 5xFAD mice (Fig. 3H). Further, there was a trend of positive but not a statistically significant association ($p = 0.081$) between sFRP1 protein levels and Aβ40 levels in the TBS-T fractions from the cortex of 5xFAD mice (Fig. 3G). Taken together, our data suggest that upregulation of sFRP1 contributes to amyloid pathology in 5xFAD mice.

Discussion

Female sex has been recognized as one of the major risks for late onset AD [17–19]. Evidently, female 5xFAD mice exhibit more severe amyloid pathology than male 5xFAD mice [20–22]. sFRP1 has recently been identified as a novel regulator of ADAM10 that contributes to AD pathogenesis [14–16]. In the present study, we show that ADAM10 levels are downregulated and sFRP1 levels are upregulated in 5xFAD mice, suggesting that both abnormal levels of ADAM10 and sFRP1 are corelate to the inhibition of the non-amyloidogenic pathway in 5xFAD mice. Importantly, we further demonstrate that there is a sex-dependent effect on sFRP1 upregulation in 5xFAD mice. Additional studies are required to determine whether sFRP1 levels in female AD patients are higher than those in male AD patients, and whether upregulation of sFRP1 contributes to more severe amyloid pathology in human AD brain and AD mouse models.

It has been reported that Aβ-correlated proteins in 5xFAD mice are generally regulated by posttranscriptional mechanisms [16]. In the present study, we demonstrate that sFRP1 protein level is more profoundly upregulated than sFRP1 mRNA in 5xFAD mice, suggesting that upregulation of sFRP1 occurs not only at the transcriptional level but also through posttranscriptional mechanisms. While there is no sex effect on sFRP 1 transcriptional levels in brains of 5xFAD mice, the levels of sFRP1 protein in female 5xFAD mice are significantly higher than those in male 5xFAD mice, indicating that the sex effect on sFRP 1 protein levels is through posttranscriptional processes in 5xFAD mice. Interestingly, the sFRP1 protein levels in female NTG mice are also significantly higher than those in male NTG mice, suggesting that sex-dependent differences of sFRP1 levels could be not associated with amyloid pathology. sFRP1 is upregulated in human AD brains [14, 16]. It

will be interesting to determine whether there is a posttranscriptional regulatory mechanism for sFRP1 upregulation in the brain of human AD patients in the future.

In summary, we have demonstrated that there is a sex-dependent effect on sFRP1 upregulation in 5xFAD mice through both transcriptional and posttranscriptional regulatory processes, although the exact mechanisms remain to be elucidated. Our findings also indicate that upregulation of sFRP1 could contribute to more severe amyloid pathology in female 5xFAD mice. sFRP1 is an important negative regulator of ADAM10, a constitutive α-secretase of AβPP. Upregulation of sFRP1 in the aged brain could result in inhibition of ADAM10 activity and consequent promotion of the pathogenic Aβ peptide generation. Therefore, it will be interesting to determine whether pharmacological inhibition of sFRP1 has therapeutic potential for AD in the future.

Acknowledgements

We thank the members of our laboratories for helpful discussions.

Fundings

This work was supported in part by grants from the National Institutes of Health R21AG065653 to YL and the Florida Department of Health Ed and Ethel Moore Alzheimer's Disease Research Program Pilot Grant 9AZ09 to YL.

Availability of Data and Material

All data generated during this study are included in this article or are available on reasonable request from the corresponding author.

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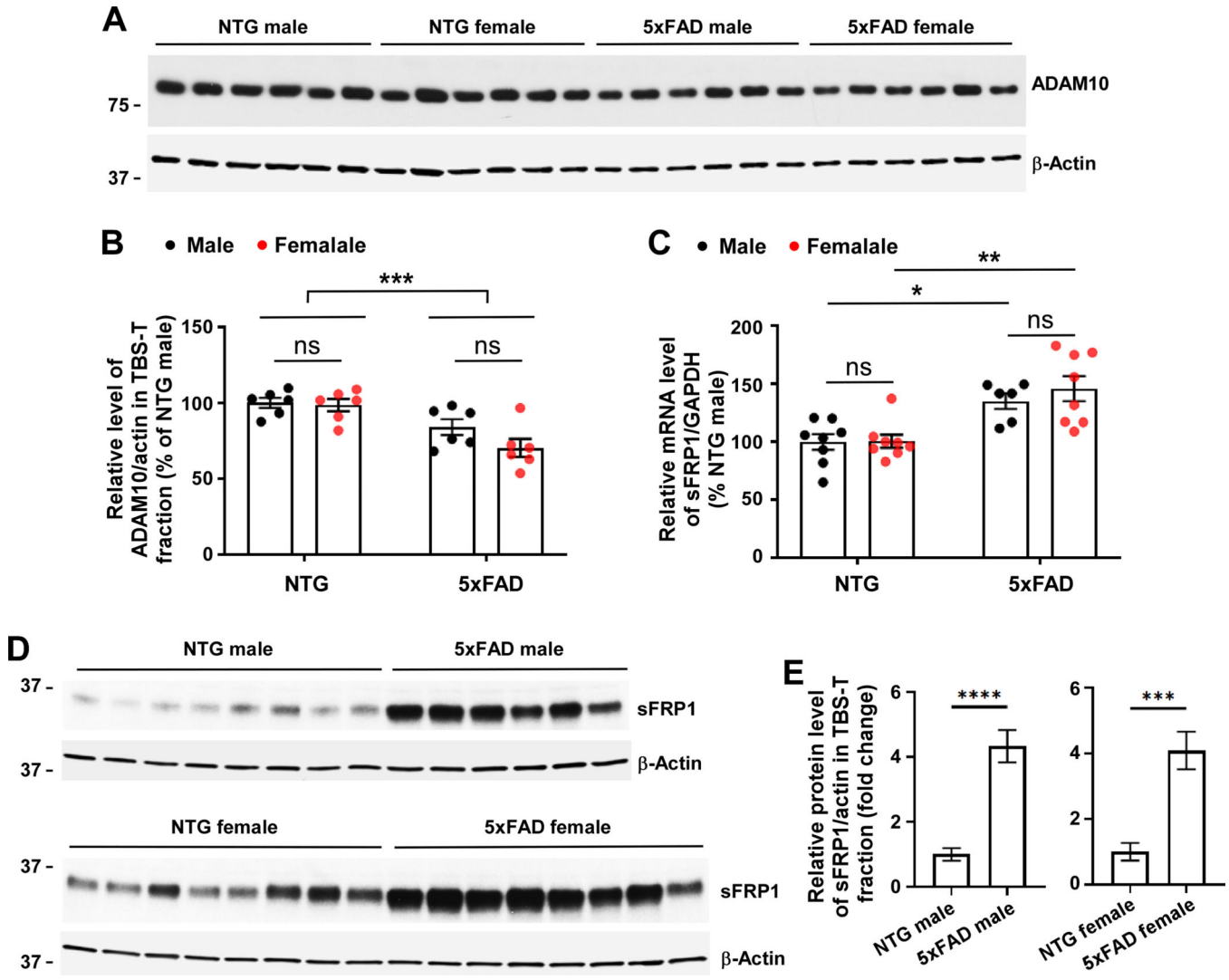

Fig. 1.

Downregulation of AMAM10 and upregulation of sFRP1 in 5xFAD mice. (**A, B**) Downregulation of ADAM10 protein levels in 5xFAD mice. The mouse brain TBS-T fractions were prepared from the cortex of 5xFAD mice and NTG littermate controls at 14 months of age. The levels of ADAM10 protein in the cortex of 5xFAD mice (6 of each sex) and NTG littermate controls (6 of each sex) were examined by Western blotting and normalized with β-actin levels. (**C**) Upregulation of sFRP1 mRNA levels in 5xFAD mice. Total RNAs were isolated from the cortex of 5xFAD mice and age-matched NTG littermate controls at 14 months of age. The levels of sFRP1 mRNA in the cortex of 5xFAD mice (6 males and 8 females) and NTG littermate controls (8 males and 8 females) were examined by quantitative qPCR. (**D, E**) Upregulation of sFRP1 protein levels in 5xFAD mice. (**D**) The mouse brain TBS-T fractions were prepared from the cortex of 5xFAD mice and NTG littermate controls at 14 months of age, and sFRP1 protein levels were examined by Western blotting and normalized with β-actin levels. (**E**) Comparison of sFRP1 levels in TBS-T fraction of male 5xFAD mice (n = 6) and male NTG mice (n = 8), and comparison of sFRP1 levels in TBS-T fractions of female 5xFAD mice (n = 8) and female NTG mice (n = 8).

Two-way ANOVA and Turkey tests or unpaired t tests. * $p < 0.05$; ** $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

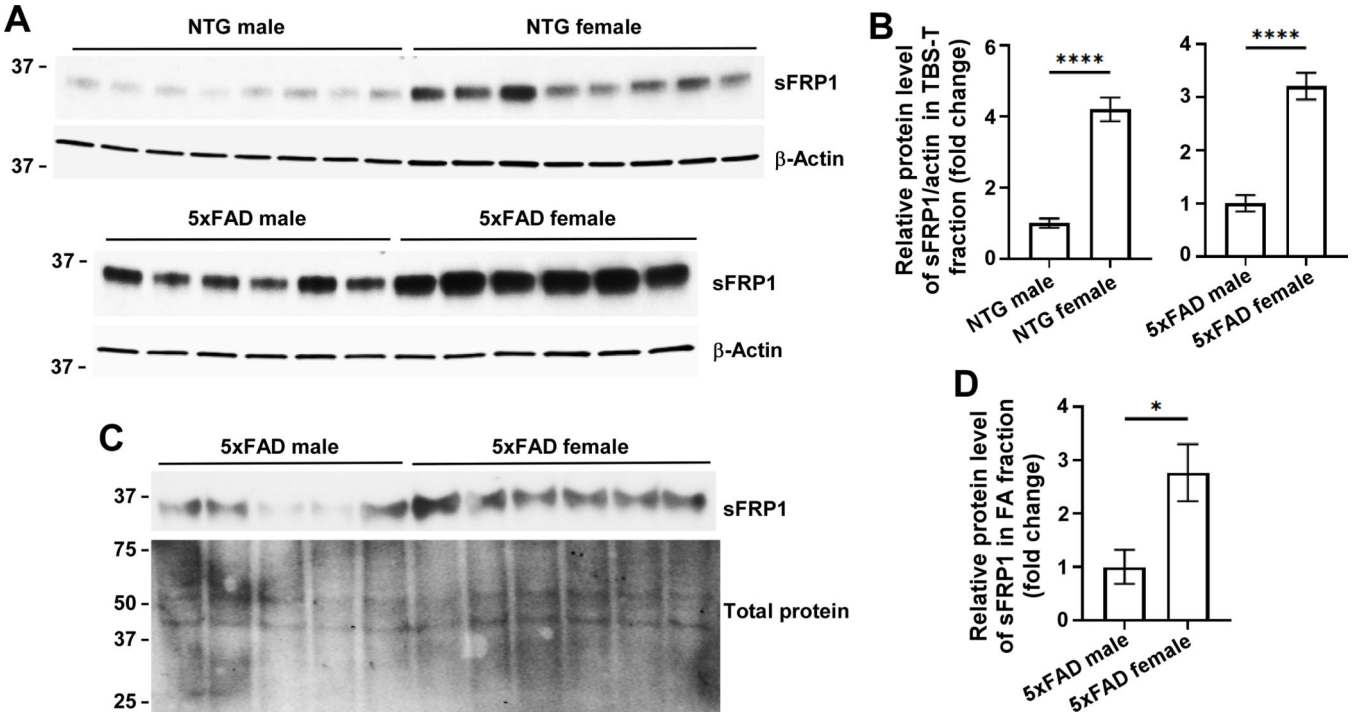

Fig. 2.

Sex-dependent upregulation of sFRP1 protein levels in 5xFAD and NTG mice. sFRP1 protein levels in the TBS-T and FA fractions from the cortex of 5xFAD mice and NTG mice at 14 months of age were examined by Western blotting. sFRP1 protein levels in FA fraction were normalized with total protein staining and quantification of two most intense bands around 50 kDa. (**A, B**) Comparison of sFRP1 levels in TBS-T fraction of male NTG mice (n = 8) and female NTG mice (n = 8), and comparison of sFRP1 levels in TBS-T fractions of male 5xFAD mice (n = 6) and female 5xFAD mice (n = 6). (**C, D**) Comparison of sFRP1 levels in FA fractions of male 5xFAD mice (n = 5) and female 5xFAD mice (n = 6). Unpaired t tests. *, $p < 0.05$; ****, $p < 0.0001$.

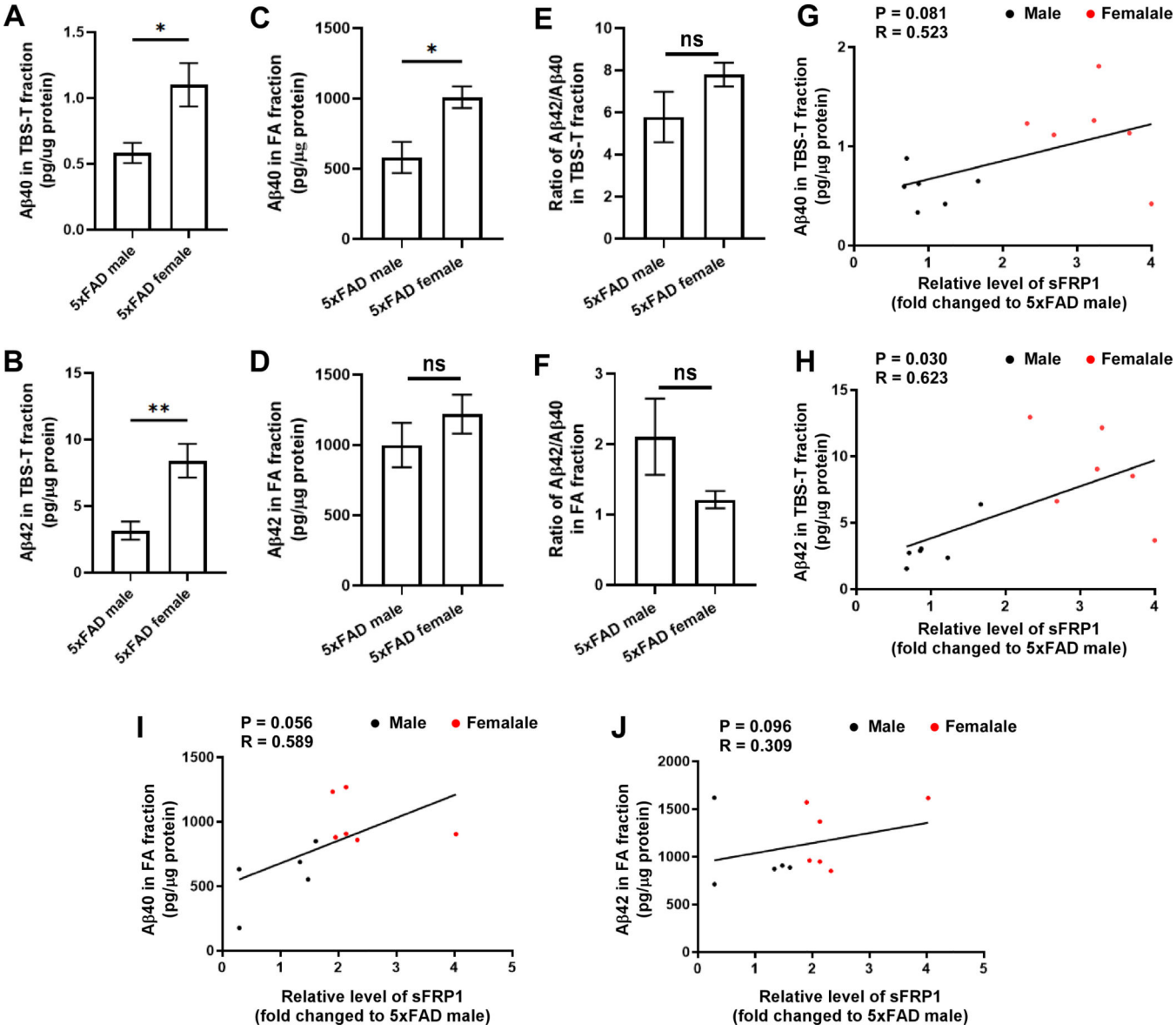

Fig. 3.

Upregulation of sFRP1 is associated with Aβ levels in the brain of 5xFAD mice. (**A-F**) Female 5xFAD mice accumulate more Aβ than males. Aβ40 and Aβ42 levels in the TBS-T and FA fractions of the cortex of 5xFAD mice at 14 months of age were determined by ELISA assays. (**A**) Comparison of Aβ40 levels in TBS-T fractions of male 5xFAD mice (n = 5) and female 5xFAD mice (n = 6). (**B**) Comparison of Aβ42 levels in TBS-T fractions of male 5xFAX mice (n= 5) and female 5xFAD mice (n = 6). (**C**) Comparison of Aβ40 levels in FA fractions of male 5xFAD mice (n = 5) and female 5xFAD mice (n = 6). (**D**) Comparison of Aβ42 levels in FA fractions of male 5xFAD mice (n =5) and female 5xFAD mice (n = 6). (**E**) Comparison of Aβ42/Aβ40 ratios in TBS-T fractions of male 5xFAD mice (n = 5) and female 5xFAD mice (n = 6). (**F**) Comparison of Aβ42/Aβ40 ratios in FA fractions of male 5xFAD mice (n = 5) and female 5xFAD mice (n = 6). Unpaired t tests. * $p < 0.05$; ** $p < 0.01$. (**G-J**) The associations between Aβ40/Aβ42 and sFRP1 in TBS-T

and FA fractions from the cortex of 5xFAD mice (5 males and 6 females) were analyzed by linear regression with the GraphPad Prism 9 software. The values of R and p are listed. (**G**) Association between Aβ40 and sFRP1 in TBS-T fractions. (**H**) Association between Aβ42 and sFRP1 in TBS-T fractions. (**I**) Association between Aβ40 and sFRP1 in FA fractions. (**G**) Association between Aβ42 and sFRP1 in FA fractions.