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Cerebral microvascular rather than parenchymal amyloid β -protein pathology promotes early cognitive impairment in transgenic mice

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Abstract

Alzheimer's disease (AD) is an age-dependent neurodegenerative condition that causes a progressive decline in cognitive function. Accumulation of amyloid β -protein (A β) in the brain is a prominent feature of AD and related disorders. The levels of A β accumulation alone are not a reliable predictor of cognitive deficits, which suggests other factors such as location of deposition may be more important. A β accumulates in AD brain in the form of parenchymal amyloid plaques and cerebral vascular deposits. Although both types of lesions can contribute to cognitive decline their temporal impact remains unclear. Moreover, cerebral microvascular pathology is identified as an early driver of cognitive impairment. Here for the first time, we compared two transgenic mouse strains, Tg-5xFAD and Tg-SwDI, which exhibit similar onset and anatomical accumulation of A β , but with distinct parenchymal and microvascular compartmental amyloid deposition, to assess the impact of their respective pathologies on cognitive impairment. Cohorts of each line were tested at 3 and 6 months of age to assess the relationship between spatial working memory and quantitative A β pathology. At 3 months of age, Tg-SwDI mice with onset of cerebral microvascular amyloid were behaviorally impaired, while the Tg-5xFAD, which had disproportionately higher levels of total A β , soluble oligomeric A β , and parenchymal amyloid were not. However, at 6 months of age, behavioral deficits for both groups of transgenic mice were evident, as the levels of A β pathologies in the Tg-5xFAD accumulated to extremely high amounts. The present findings suggest that early-onset cerebral microvascular amyloid deposition, that precedes high parenchymal levels of A β , may be an important early factor in the development of cognitive deficits. Key words: amyloid β protein; cerebral microvascular, parenchymal, pathology, cognitive impairment, transgenic mice

Keywords

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INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disorder and leading cause of dementia, is pathologically characterized by accumulation of extracellular amyloid β protein ($A\beta$) deposits and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein [1,2]. In AD, $A\beta$ deposition occurs as parenchymal plaques and in cerebral blood vessels, the latter condition known as cerebral amyloid angiopathy (CAA). Although some studies have found a correlational relationship between cerebral $A\beta$ levels and dementia in AD [3–8], others have reported that the amount of $A\beta$ levels alone are not a reliable predictor of cognitive deficits [9–12]. This suggests that subtle aspects associated with $A\beta$, such as its specific compartmental accumulation, may be more important determinants related to the onset of dementia. There is evidence that both parenchymal and cerebral microvascular $A\beta$ deposition can promote neuroinflammation and cognitive deficits in patients with AD and related disorders [13–17]. However, emerging studies have underscored the importance of cerebral vascular disease, including CAA, as a potential early driver of cognitive impairment independent of parenchymal $A\beta$ pathology [18–26]. Yet, it is not known how the specific accumulation of $A\beta$ in the cerebral microvascular and the parenchymal compartments temporally contributes to the cognitive decline in patients.

Transgenic mice that specifically develop cerebral microvascular or parenchymal amyloid deposition provide an opportunity to address the contribution of each pathological feature to cognitive impairment. On the one hand, Tg-SwDI mice, a model of early-onset and extensive cerebral microvascular $A\beta$ deposition, have shown that capillary amyloid formation and associated neuroinflammation is intimately linked to behavioral impairments [27–29]. On the other hand, Tg-5xFAD mice, a model of early-onset and robust parenchymal amyloid accumulation exhibit plaque-associated neuroinflammation and develop early behavioral impairments [30–32]. Although both of these distinct transgenic mouse models have been shown to develop impairments in spatial learning and memory tasks the comparative timing of these deficits and their relationship to their respective $A\beta$ pathologies is poorly understood.

Here for the first time, we employed cohorts of these two transgenic lines in parallel to determine the onset of behavioral deficits and the relationship of the deficits to the levels of $A\beta$, soluble $A\beta$ oligomers and the spatial accumulation of fibrillar amyloid. At three months of age Tg-SwDI mice with the onset of cerebral microvascular amyloid, but not Tg-5xFAD mice with the onset of parenchymal amyloid, exhibited deficits in spatial learning memory despite having much lower levels of total $A\beta$, soluble oligomeric $A\beta$, and overall fibrillar amyloid deposition.

MATERIALS AND METHODS

Animals

All work with mice followed National Institutes of Health guidelines and was approved by the Stony Brook University Institutional Animal Care and Use Committee (IACUC). Tg-SwDI mice were generated and characterized in our laboratory as previously described [27–29]. Tg-SwDI mice express the human Swedish/Dutch/Iowa $A\beta$ PP transgene in neurons

under control of the Thy1 promoter element and develop early-onset A β deposition and cerebral microvascular amyloid accumulation in the cortex, thalamus and subiculum. Tg-5xFAD mice were obtained from Jackson Laboratories. Tg-5xFAD mice express the human Swedish/Indiana mutant A β PP transgene in neurons also under control of the Thy1 promoter element and develop early-onset A β deposition and, in this case, parenchymal plaque amyloid accumulation in the cortex, thalamus and subiculum [30]. Six to eight heterozygous Tg-SwDI, heterozygous Tg-5xFAD, and wild-type mice were used for each 3 and 6 month timepoints.

Behavioral testing

Barnes maze testing was performed as described [29]. Briefly, the Barnes Maze apparatus, originally developed for rats [33], consisted of a sealed wooden disk 91 cm in diameter, elevated 75 cm off the floor, with eight escape holes, spaced 24.5 cm apart. An escape box measuring 10 cm $\times$ 8.5 cm $\times$ 4 cm was used. Visible distal cues were placed around the room and remained constant throughout each day of testing. Each mouse was given 2 trials per day with a 15 min inter-trial interval. Testing spanned 5 consecutive days. An observer sat 1.5 m away from the maze during trials. At the start of each trial, each mouse was picked up from their home cages by the base of their tail and placed onto the center of the maze. A timer was then started and the mouse was allowed a maximum of 5 min to find a randomized designated hole and escape box. When the mouse entered the box, the timer was stopped and they were given 1 min inside the box before being transferred back to the home cage. If the mouse did not find the escape hole within the 5 min limit, they were picked up by the base of their tail and placed into the hole and left there for 1 minute. The animals were also assessed in the wire hang, rotorod, light/dark box, 0-maze and digiscan activity monitor as previously described [29], following testing in the Barnes maze (data not shown).

Tissue preparation

Mice were overdosed with 2.5% avertin and intracardially perfused with PBS and the brains were removed and bisected along the midsagittal plane. One hemisphere was snap frozen and used for ELISA and protein analysis. The other hemisphere was placed in 70% ethanol, followed by xylene treatment and embedding in paraffin for immunohistochemical and histological analyses as described [27–29].

Immunochemical analysis of cerebral A β peptides

The pools of A β ₄₀ and A β ₄₂ were determined by using a specific ELISA as previously described [34,35]. Briefly, soluble A β ₄₀ and A β ₄₂ levels were determined in mouse forebrain tissues extracted with 0.1 M sodium carbonate, 0.05 M NaCl, pH 11.5 containing protease inhibitor cocktail. The insoluble A β ₄₀ and A β ₄₂ levels were determined in the insoluble pellets resulting from the carbonate extracted brain tissues that were subsequently extracted with 5 M guanidine-HCl. In the sandwich ELISAs, A β ₄₀ and A β ₄₂ were captured using their respective carboxyl terminus-specific antibodies m2G3 and m21F12, and biotinylated antibody m3D6, specific for human A β , was used for detection [27,28,34,35]. Total A β ₄₀ and A β ₄₂ levels were determined by combining the soluble and insoluble levels of each form.

To determine the relative levels of soluble A β oligomers the soluble brain fractions prepared above were analyzed by two different methods. First, a sandwich ELISA was performed using m3D6 to capture A β species and biotinylated m3D6 for detection of any sized oligomer [35,36]. Alternatively, soluble A β oligomers were analyzed by quantitative dot blot analysis using the polyclonal anti-oligomer antibody OC11 as described [30].

Immunohistochemical analysis

Immunohistochemistry was performed as previously described [27–29,37]. Briefly, paraffin sections were cut in the sagittal plane at 10 μ m thickness using a microtome. Slides were deparaffinized by immersing in xylene and rehydrated with decreasing concentrations of ethanol. Antigen retrieval was conducted via 5 min incubation with proteinase K (0.2 mg/ml) at 22° C. Primary affinity purified rabbit polyclonal antibody that recognizes residues 1–15 of human A β (1:200; [38]) was detected with horseradish peroxidase-conjugated secondary antibody and visualized with a stable diaminobenzidine solution (Life Technologies, Grand Island, NY) as substrate. Sections were counterstained with hematoxylin. Alternatively, deposited fibrillar amyloid was detected with thioflavin-S staining and cerebral vessels were detected with a primary antibody to collagen IV (1:100, Research Diagnostics Inc., Flanders, NJ) and an Alexa Fluor 594-conjugated secondary antibody. Histological images were captured with an Olympus DP72 camera attached to an Olympus BX60 microscope and a Dell desktop computer.

Quantitative analysis of regional A β deposition and microvascular CAA

Total A β and fibrillar amyloid burden in the regions of the cortex, thalamus and subiculum was respectively quantified on the same set of systematically sampled immunostained or thioflavin-S stained sections, respectively, using NIH image J 1.32 software. The percentage of thioflavin-S labeled blood vessels in the same fields as above was determined with using stereological principles as described [39].

Statistical analyses

All biochemical and pathological data were compared between Tg-SwDI mice and Tg-5xFAD mice using *t* test with significance considered at $p < 0.05$. Repeated measures ANOVAs were used to analyze data in the Barnes maze.

RESULTS

Selection of mouse models

In the present study we sought to determine the onset of cognitive impairments associated with either cerebral microvascular amyloid pathology or parenchymal amyloid pathology using two distinct human A β PP transgenic mouse models: Tg-SwDI and Tg-5xFAD, respectively. These models were chosen for several compelling reasons. First, both models express human A β PP under control of the Thy1 promoter and, therefore, produce A β PP and A β peptides in the same sets of neurons in brain [22,25]. Second, Tg-SwDI and Tg-5xFAD mice develop their respective pathologies in similar neuroanatomical regions: cortex, thalamus, subiculum, and hippocampus. Lastly, there is a similar onset of about two to three months of age for the respective pathologies in each model. Together, these features of Tg-

SwDI mice and Tg-5xFAD mice provide an excellent opportunity to longitudinally compare the onset and extent of cerebral microvascular amyloid pathology or parenchymal amyloid pathology as it relates to the onset of cognitive impairment.

Early cognitive impairments in Tg-SwDI mice but not Tg-5xFAD mice

The findings from the behavioral experiments are summarized in Fig. 1. Repeated measures ANOVA with genotype and age entered as between subject variables and testing day as a within-subject variable revealed significant main effects of genotype ($F(2,33) = 7.3, p < 0.002$), age of testing ($F(1,33) = 4.8, p < 0.03$), and a significant genotype $\times$ age interaction ($F(2,33) = 5.1, p < 0.01$). Inspection of the figure shows that at three months of age Tg-SwDI mice are impaired in escape box acquisition consistent with previous findings [29]. In contrast, Tg-5xFAD mice did not exhibit impaired acquisition of the Barnes maze (the latency to find the hidden escape box) at the three month time point. However, impairment in Barnes maze learning is strikingly evident in Tg-5xFAD mice at six months.

Tg-5xFAD mice accumulate higher levels of A β compared to Tg-SwDI mice

Since Tg-SwDI mice develop an earlier onset of cognitive impairment than Tg-5xFAD mice we next determined if this was related to amount of A β that accumulates in the brains of these mice. ELISA analysis was performed to measure the levels of A β_{40} and A β_{42} in both soluble and insoluble brain fractions. As shown in Fig. 2A, at three months of age Tg-SwDI mice accumulated more A β_{40} than A β_{42} with higher amounts in the insoluble fraction, consistent with previous findings [27,29,36]. In contrast, Tg-5xFAD mice accumulated higher levels of A β_{42} than A β_{40} , consistent with the presence of the FAD-linked I716V and V717I A β PP mutations and M146L and L286V PS1 mutations present in this model [30]. However, the amounts of both A β_{40} and A β_{42} in Tg-5xFAD mice were much higher than in Tg-SwDI mice. Although the amounts of each A β peptide sharply increased at six months of age the ratios of A β_{40} and A β_{42} in each respective mouse line remained similar and the disproportionately higher levels in Tg-5xFAD mice remained evident (Fig. 2B).

Soluble A β oligomers have been implicated as important peptide assemblies that can promote neuronal dysfunction [39–42]. Therefore, we next determined the levels of soluble A β oligomers in Tg-SwDI mice and Tg-5xFAD mice using two different techniques. First, we used a sandwich ELISA technique to measure soluble oligomers in the soluble brain fraction [35,36]. Alternatively, we performed quantitative dot blot analysis on soluble brain fractions using the A β oligomer-specific antibody OC11 [36,43]. Both of these complimentary approaches revealed that at three months of age Tg-5xFAD mice possessed extremely high levels of soluble A β oligomers compared to Tg-SwDI mice (Fig. 3A,B). These same techniques showed that at six months of age the levels of soluble A β oligomers increased in both mouse lines but, again, the amounts were markedly higher in Tg-5xFAD mice (Fig. 3C,D). Together, these findings show that at three months of age Tg-5xFAD mice accumulate much higher levels of A β and soluble A β oligomers compared to Tg-SwDI mice suggesting that these measures are not well correlated with cognitive impairment.

Tg-5xFAD mice deposit higher amounts of A β compared Tg-SwDI mice

Although the quantitative measures above indicate that Tg-5xFAD mice accumulated much higher amounts of A β than Tg-SwDI mice we next determined if there was a different neuroanatomical pattern of total A β deposition. At three months of age immunostaining for total A β in brain tissue sections showed that Tg-5xFAD mice deposit higher amounts compared to Tg-SwDI mice (Fig. 4), consistent with the ELISA measurements of insoluble A β (Fig. 2). Quantitation of regional A β deposition showed Tg-5xFAD mice had significantly higher amounts in the cortex and thalamus, although the levels in the subiculum were similar (Fig. 4C). At six months of age it was evident that A β deposition increased in both mouse lines although it again was more robust in Tg-5xFAD mice. Quantitation showed that the significantly higher amounts of deposited A β in the cortex and thalamus of Tg-5xFAD mice remained although the amount of subicular A β was higher in Tg-SwDI mice (Fig. 4F). These results indicate that early on the amount of total A β deposition is higher in Tg-5xFAD mice compared to Tg-SwDI mice, but again does not correlate with the onset of cognitive impairment.

Early-onset cerebral microvascular amyloid accumulation correlates with early cognitive impairment in Tg-SwDI mice

The experiments above focused on the quantitative neuroanatomical distribution of total A β deposition (Fig. 4). We next sought to determine the amounts and distribution pattern of deposited fibrillar amyloid in the two mouse lines. Brain tissue sections were stained with thioflavin S to identify fibrillar amyloid and immunolabeled to identify cerebral blood vessels. At three months of age Tg-SwDI mice begin to accumulate fibrillar amyloid that is restricted to the microvessels (Fig. 5A) whereas Tg-5xFAD mice start to accumulate parenchymal plaque fibrillar amyloid (Fig. 5B). Quantitation of total amyloid deposition shows higher amounts in Tg-5xFAD mice compared to Tg-SwDI mice (Fig. 5C). This reflects that the cerebral microvascular fibrillar amyloid in Tg-SwDI mice presents as small, punctate deposits whereas the parenchymal plaque amyloid deposits in Tg-5xFAD mice are much larger. However, the measurement exclusively of cerebral microvascular amyloid shows that it is absent in Tg-5xFAD mice and is highest in the subiculum region of Tg-SwDI mice (Fig. 4D). Although the total amyloid burden increases in both lines of mice at six months and remains significantly higher in Tg-5xFAD mice, the distribution pattern remains the same with fibrillar amyloid restricted to cerebral microvessels in Tg-SwDI mice and largely parenchymal plaques in Tg-5xFAD mice (Fig. 4E–H). These findings suggest that it is not the total fibrillar amyloid burden, but rather the specific early-onset accumulation of fibrillar cerebral microvascular amyloid that correlates as an early driver of cognitive impairment.

DISCUSSION

It has been proposed that the accumulation of A β in the brain promotes deleterious downstream effects such as neuroinflammation, hyperphosphorylation of tau and neuronal death, which in turn causes the cognitive dysfunction seen in AD [1,2]. However, A β can spatially accumulate in the brain as parenchymal plaques or cerebral vascular deposits, both of which can contribute to cognitive impairment. The main objective of the present study

was to use, in parallel, two distinct transgenic mouse models of either parenchymal amyloid (Tg-5xFAD) or cerebral microvascular amyloid (Tg-SwDI) deposition to investigate the temporal contribution of each type of pathology to cognitive impairment. Contrary to a simple positive relationship between increasing levels of total A β , soluble oligomeric A β , or deposited A β , our data suggest that the site of fibrillar A β accumulation may be a more important causal factor in the initial cognitive decline in these animals, and perhaps also in AD patients. Our findings show that overall A β levels, while associated with cognitive deficits when reaching extremely high levels, do not have a particularly strong correlation with emerging cognitive decline. While Tg-5xFAD mice exhibited much higher levels of both soluble and insoluble A β_{40} and A β_{42} peptides, as well as soluble A β oligomers, in both the three and six month age groups compared to Tg-SwDI mice, they were largely unimpaired in the Barnes maze learning task at the earlier age. These observations point to the importance of the spatial deposition of A β as another significant factor in promoting cognitive decline. Indeed, Tg-5xFAD mice and Tg-SwDI mice had markedly distinct parenchymal amyloid versus microvascular amyloid deposition patterns, respectively.

Our data show a striking difference between the Tg-5xFAD and Tg-SwDI strains regarding the amount of accumulated A β . At three months or six months of age Tg-5xFAD mice accumulate much more total A β than Tg-SwDI mice. Notably, at either age the amount of A β_{42} is $\approx$ 6-fold higher than A β_{40} in Tg-5xFAD mice whereas in Tg-SwDI mice it is reversed with the amount of A β_{40} $\approx$ 8-fold higher than A β_{42} (Figure 2). Generally, A β_{42} is considered to be more pathogenic due to its stronger ability to assemble into toxic species compared to A β_{40} [1,2,44,45]. However, specific mutations in A β , including the Dutch E22Q and Iowa D23N substitutions that are associated with familial forms of CAA [46–48], exhibit greatly enhanced fibrillogenic and pathogenic properties compared to the normal, wild-type forms of A β and strongly target cerebral vascular amyloid formation [49–53].

In either case, monomeric A β peptides initially aggregate as low molecular mass oligomeric species that adopt progressive β -sheet content to assemble into higher oligomeric forms, protofibrils, and ultimately amyloid fibrils that deposit in cerebral tissues [44,45,54–56]. It is likely that different assemblies of A β can promote various pathogenic responses that collectively contribute to the syndrome of AD. For example, different soluble oligomeric species of A β are directly toxic to neurons, can interfere with long-term potentiation, and disrupt the integrity of cell membranes [39–42,44,45,56]. On the other hand, fibrillar assemblies of A β are toxic to neuronal and cerebral vascular cells, can activate complement, and can stimulate potent neuroinflammatory responses [57–62]. However, we found at either age the amount of soluble A β oligomers was vastly higher in Tg-5xFAD mice compared to Tg-SwDI mice. The absence of cognitive impairment in three months old Tg-5xFAD mice indicates that the comparatively high level of soluble A β oligomers is not well correlated with the onset of these deficits.

Although the onset of cerebral A β deposition occurs on a similar time frame and in the same neuroanatomical regions in Tg-SwDI mice and Tg-5xFAD mice the extent of cerebral A β deposition is generally more severe in Tg-5xFAD mice (Fig. 4). Likewise, the amount of fibrillar amyloid deposition is also more severe in Tg-5xFAD mice indicating that total amyloid burden alone does not correlate with early-onset cognitive impairment. However,

the compartmental accumulation of fibrillar amyloid is distinct between Tg-5xFAD mice, with its primarily parenchymal plaque deposition, and Tg- SwDI mice, with its cerebral microvascular deposition (Fig. 5). These results indicate that the very high A β burden and parenchymal amyloid load seen in Tg-5xFAD mice is insufficient to promote deficits in spatial learning and memory whereas the much lower A β levels and focal cerebral microvascular amyloid found in Tg-SwDI mice was adequate to impair performance.

There is growing evidence that vascular-mediated brain injury through ischemic strokes, microbleeds and white matter damage can be early contributing factors to cognitive impairment [63–69]. Certainly microvascular accumulation of amyloid is another form of vascular-mediated brain injury that can have similar deleterious consequences to cognition. In the context of amyloid deposition, previous reports support the idea that the presence of cerebral vascular amyloid is associated with a worse cognitive outcome compared to parenchymal amyloid deposition alone and that this is particularly prominent in the earliest stages of AD related dementia [71–73]. In conclusion, our results show that in transgenic mouse models parenchymal A β and amyloid accumulation does not drive cognitive dysfunction until a high threshold is reached whereas cerebral microvascular amyloid has an earlier influence on cognitive decline. Cerebral vascular amyloid and associated pathologies may be more efficacious targets for the onset of amyloid-associated cognitive impairment.

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Figure 1. Measurement of cognitive impairments in Tg-SwDI mice and Tg-5xFAD mice
Groups of three month old and six month old wild-type mice, Tg-SwDI mice, and Tg-5xFAD mice were tested for spatial learning and memory in the Barnes maze task. Tg-SwDI mice showed impairment at three months and six months whereas impairment was not evident in Tg-5xFAD mice until six months. Data are the mean $\pm$ SD of six mice per group. ○, wild-type mice, 3 months; ○, Tg-5xFAD mice, 3 months; ○, Tg-SwDI 3 months; ●, wild-type mice, 6 months; ●, Tg-5xFAD mice, six months; ●, Tg-SwDI mice, 6 months.

Figure 2. Measurements of cerebral Aβ levels in Tg-SwDI mice and Tg-5xFAD mice
 ELISA measurements for Aβ peptides were performed on forebrain hemispheres from three months old and six months old Tg-SwDI mice and Tg-5xFAD mice. These results showed that the levels of soluble and insoluble Aβ40 and Aβ42 peptides were dramatically higher in Tg-5xFAD mice compared to Tg-SwDI mice. ELISA data shown are mean ± S.D. (n = 8–10 animals per group). * $p < 0.0001$.

Figure 3. Measurements of cerebral soluble A β oligomer levels in Tg-SwDI mice and Tg-5xFAD mice
 ELISA measurements for A β oligomers were performed on the soluble fractions of forebrain hemispheres from three months old (**A**) and six months old (**B**) Tg-SwDI mice and Tg-5xFAD mice. ELISA data shown are the mean $\pm$ S.D. (n = 8- 10 animals per group). * p < 0.0001. Representative dot blot measurements for A β oligomers were performed on the soluble fractions of forebrain hemispheres from three months old (**C**) and six months old (**D**)

Tg-SwDI mice and Tg-5xFAD mice. These results show that Tg-5xFAD mice have much higher levels of soluble A β oligomers compared to Tg-SwDI mice.

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Figure 4. Cerebral Aβ deposition in Tg-SwDI mice and Tg-5xFAD mice forebrain tissue
Human Aβ deposits were detected by immunostaining (brown) in the forebrain of three months and six months old Tg-SwDI mice (A,D) and Tg-5xFAD mice (B,E). Scale bars = 1 mm. Quantitation of regional Aβ deposition in three months old (C) and six months old (F) Tg-SwDI mice and Tg-5xFAD mice. Data shown are the mean ± S.D. (n = 8 animals per group). * $p < 0.0001$; ** $p < 0.0004$. These results show that Tg-5xFAD mice higher levels of cerebral Aβ deposition compared to Tg-SwDI mice.

Figure 5. Fibrillar amyloid deposition in Tg-SwDI mice and Tg-5xFAD mice
Brain sections from Tg-SwDI mice and Tg-5xFAD mice at three months old (A,B) and six months old (E,F) were stained for fibrillar A β using thioflavin-S (green) and immunolabeled for collagen type IV to identify cerebral microvessels (red). Scale bars = 50 μ M. These results show that Tg-SwDI mice develop cerebral microvascular fibrillar amyloid whereas Tg-5xFAD mice develop parenchymal fibrillar amyloid plaques. Quantitation of total thioflavin-S positive amyloid load in different brain regions of three months old (C) and six months old (G) Tg-SwDI mice and Tg-5xFAD mice. Data shown are mean $\pm$ S.D (n = 8)

animals per group). $*p < 0.0001$. These results show that Tg- 5xFAD mice accumulate higher levels of total fibrillar amyloid. Quantitative stereological estimation of cerebral microvascular amyloid load in different brain regions of three months old (**D**) and six months old (**H**) Tg-SwDI mice and Tg-5xFAD mice. Data shown are mean $\pm$ S.D ($n = 8$ animals per group). Only Tg-SwDI mice exhibit cerebral microvascular amyloid.

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