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ORIGINAL RESEARCH

Increased Human Wildtype Tau Attenuates Axonal Transport Deficits Caused by Loss of APP in Mouse Models

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Abstract: Amyloid precursor protein (APP) is implicated in axonal elongation, synaptic plasticity, and axonal transport. However, the role of APP on axonal transport in conjunction with the microtubule associated protein tau continues to be debated. Here we measured *in vivo* axonal transport in APP knockout mice with Manganese Enhanced MRI (MEMRI) to determine whether APP is necessary for maintaining normal axonal transport. We also tested how overexpression and mutations of tau affect axonal transport in the presence or absence of APP. *In vivo* axonal transport reduced significantly in the absence of functional APP. Overexpression of human wildtype tau maintained normal axonal transport and resulted in a transient compensation of axonal transport deficits in the absence of APP. Mutant R406Wtau in combination with the absence of APP compounded axonal transport deficits and these deficits persisted with age. These results indicate that APP is necessary for axonal transport, and overexpression of human wildtype tau can compensate for the absence of APP at an early age.

Keywords: manganese enhanced MRI, amyloid precursor protein (APP), tau, axonal transport

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Introduction

The presence of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau in Alzheimer's Disease (AD) pathology has resulted in numerous studies designed to elucidate their role and potential interaction. Multiple reports indicate that Aβ pathology precedes the accumulation of hyperphosphrylated tauin AD, leading to the focus on Aβ as a key target for treatment.[1,2] However, it has also been shown that reduced expression of tau results in decreased Aβ neuro- and excitotoxicity.[3,4] These reports indicate that an interaction between Aβ and tau may be requisite for the development of AD pathology. One specific area of potential interaction between these two proteins is within the axon, as there is increasing evidence that the aberrant accumulation of both Aβ and tau may impede axonal transport early in disease progression.[5]

Aβ results from the aberrant cleavage of Amyloid precursor protein (APP). APP is a type I transmembrane protein that is normally involved in neuronal outgrowth, synaptogenesis, axonal targeting and function, cell adhesion, vesicular transport, and insulin regulation.[6,7] However, the biological necessity of APP is still being assessed. APP knockout (APP –/–) mice are capable of reproducing and initially appear to have normal brain morphology as determined with hematoxylin and eosin staining but do exhibit reduced weight gain, reduced forelimb grip, and increased gliosis in learning and memory centers of the brain indicating impairment in the CNS.[8] One report on APP –/– mice described changes in protein localization at proximal ends of sciatic nerve ligations, but not overall protein levels suggesting decreases in axonal transport rates occurs in the absence of APP.[9] A separate study reported that APP was not required for the transport of some proteinsbringing to questions the role of APP within the axon.[10]

One study in *D. melanogaster* reported that with the APP homologue amyloid precursor protein-like protein (APPL) knocked out, axonal swellings are present in neurons, suggesting that APPL is necessary for axonal transport.[11] Other studies show APP involvement in vesicular transport and that loss of APP is associated with abnormalities in both pre- and postsynaptic structures.[12] Loss of APP has also been shown to result in aberrant increases in phosphorylation of tau implicating a deficit in microtubule stability.[13]

The observation that disruptions in normal APP may affect tau phosphorylation further supports the hypothesis that changes in APP could impair axonal transport.[13,14] Specifically, tau primarily localizes to the axon[15,16] where it is known to bind to and stabilize microtubules in a dynamic process controlled through phosphorylation.[15,17–19] Hyperphosphorylation of tau ultimately leads to the development of NFTs in AD and also contributes to several other neurodegenerative diseases known collectively as tauopathies. Furthermore, it has been demonstrated thathyperphosphorylated tau adversely affects axonal transport.[20] One particular mouse model of the tauopathy fronto-temporal dementia with parkinsonism linked to chromosome 17 is linked to the tau mutation, R406W. This mutation reportedly reduces the axonal transport of tau.[21] Alternatively, the transgenic overexpression of human wildtype tau in mouse models exhibits normal axonal transport ratesindicating that an increased amount of normal tau protein does not harm axonal function.[21,22]

Clearly, APP may play a normal role in the regulation of tau. We hypothesized that loss of APP function would result in axonal transport deficits in mice, and that this transport deficit would persist in combination with additional levels of normal or mutant tau due to the evidence that APP dysfunction in disease likely promotes tau dysfunction. Utilizing Manganese Enhanced MRI (MEMRI) for the *in vivo* measure of axonal transport, we found that young and aged APP –/– mice exhibit axonal transport deficits. Overexpressing human wildtype tau ameliorated the APP –/– effect on axonal transport, while mutated tau exacerbated APP –/– axonal transport deficits. The work shown here demonstrates that APP normally affects axonal transport that can be modulated with the addition of normal or mutant tau. Together, these data implicate an interaction between APP and tau that ultimately impactsaxonal transport.

Materials and Methods

Mouse Models. All mice used in this study were designed on the C57Bl/6 J background. APP –/– mice lacking the promoter and first exon of *APP* have been described previously.[8] Transgenic mice expressing the longest

isoform of wildtype human tau (APP +/+ hWTtau) and human tau bearing the R406W mutation (R406Wtau), both under the control of the αCaMKII promoter, were kindly provided by Akihiko Takashima (RIKEN Brain Science Institute, Wako, Saitama, Japan).[23,24] To generate the mice for this study, we first bred APP –/– mice with APP +/+ hWTtau or R406Wtau mice to create APP +/– hWTtau, APP +/– R406Wtau, and APP +/– offspring. Non-sibling pairs were then mated to produce the following littermates: APP +/+, APP +/+ hWTtau, APP +/+ R406Wtau, APP –/–, APP –/– hWTtau, and APP –/– R406Wtau. Genotypes were determined with the previously described PCR protocols.[8,23,24] The groups of young animals were imaged at 2–4 months (mos) of age and then separate groups of aged animals were imaged at 12–17 mos of age.

In vivo axonal transport measurements by MEMRI

Axonal transport was measured *in vivo* utilizing Manganese Enhanced MRI (MEMRI) following a modification of a previously described paradigm.[25]

Mn^{2+} Administration: Animals under isoflurane anesthesia received a nasal lavage of 4 µl of 0.75 g/ml $MnCl_2$ (Sigma, St. Louis, MO) dissolved in nanopure water. Animals awoke and recovered on a heating pad for 45 minutes post lavage. Anesthesia was induced with 5% isoflurane and maintained with 2% isoflurane in 100% O_2. Animals were placed in the prone position on a Bruker mouse holder (Bruker BioSpin, Billerica, MA). Respiration was monitored with a pressure pad placed under the animal while temperature was monitored by the use of a rectal probe and maintained at 37 °C via an air heating system (SA Instruments, Inc, Stony Brook, NY).

MRI

Images were acquired utilizing a 9.4T, Bruker Avance Biospec Spectrometer, 21 cm bore horizontal scanner with a 35 mm volume resonator (Bruker BioSpin, Billerica, MA). The imaging parameters to acquire olfactory multi-spin/multi-echo 2D MEMRI images were as follows: TR = 500 ms; TE = 10.2 ms; FOV = 3.0 cm; slice thickness = 1 mm; matrix = 128 × 128; NEX = 2; number of cycles = 15; each cycle took approximately 2 min 8 sec to acquire using Paravision software version 4 (Bruker BioSpin, Billerica, MA). The inplane resolution was 234 µm. All animal experiments were conducted in accordance with the Baylor College of Medicine Institutional Animal Care and Use Committee Requirements.

MRI analysis

The region of interest (ROI) was determined as previously reported (Smith et al 2007). Briefly, the ROI was placed on an axial slice 1 mm in front of the posterior of the olfactory bulb (OB). The ROI measured 0.23 × 0.23 mm and was vertically centered on the dorsal olfactory neuronal layer (ONL) which is comprised of olfactory receptor neuron afferents.[26] The ROI was determined by measuring the length of the olfactory bulb, locating the midpoint of this line, then extending this midpoint out to the ONL using a 90 ° angle. The pixel closest to the midpoint within 5% error was established as the ROI for all images[26]. This method of defining the ROI ensures that the widest point of the olfactory neuronal layer is considered in the measurement demonstrated in Figure 1 A–D. This ROI was copied for each cycle and each ROI value normalized to the unaffected muscle of the same slice. The small region of interest collected in this study is representative of a single fascicle of axons projecting into the olfactory neuronal layer.[27] The axonal transport rate was calculated as the change in signal intensity over time (min) (Δ in SI/ Time (min)) and then converted to percent of control with control being equal to WT mice. Statistical analyses of results are reported as mean ± SEM. Data was analyzed with two-sample t-tests for pairwise comparisons and analysis of variance for multiple comparisons followed by Newman-Keuls post-tests for significance and student t-tests for *P*-value. All statistics were calculated and prepared using Prism (GraphPad Software, Inc, La Jolla, CA).

Results

We assessed both young and aged APP –/– mice to determine whether any existing axonal transport deficits occurred during development or as a result of aging (Fig. 1). We found that at 2–4 months of age axonal transport rates are reduced by 50% in APP –/– mice (*$P = 0.0464$) compared to WT mice as shown in Figure 1E, indicating that APP is necessary for normal *in vivo* axonal transport rates in young animals. The

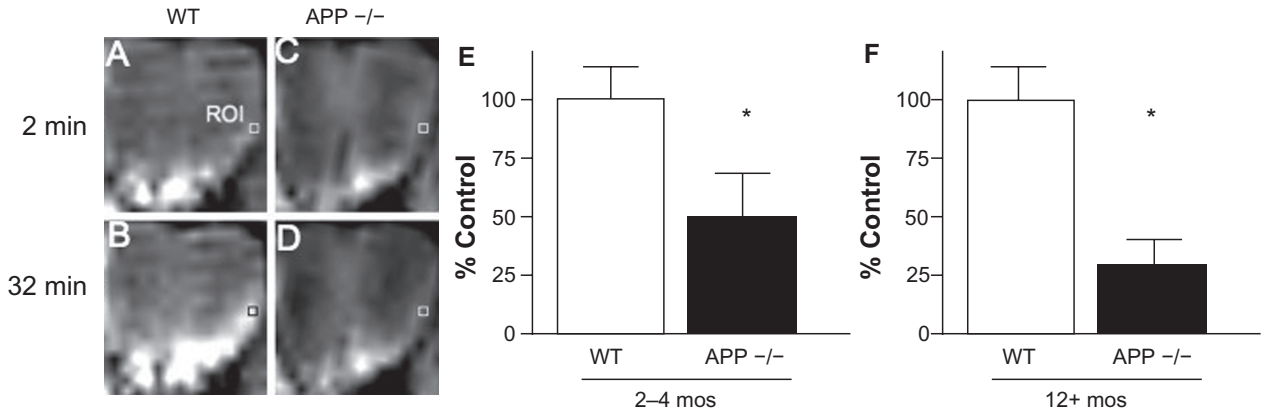


Figure 1. APP –/– axonal transport deficits occur in young and aged groups. **A–D**) Olfactory bulb images demonstrating the increase in SI in the ROI in WT animals after 30 min (**A–B**) and the lack of change in SI in the ROI in the APP –/– (**C–D**). **E**) 2–4 mos APP –/– mice have a 50% reduction in axonal transport. (WT N = 10; APP –/– N = 9; t-test **P* = 0.0464). **F**) 12+ mos (12–17 mos) APP –/– have a 70% reduction in axonal transport APP (WT N = 13; APP –/– N = 11; t-test ***P* = 0.0013). **Note:** *indicates statistical significance in the means.

aged (12–17 mos) APP –/– mice further demonstrated a 70% reduction in axonal transport rates compared to WT (***P* = 0.0012, Fig. 1F). These data demonstrate the necessity of APP for normal axonal transport *in vivo*.

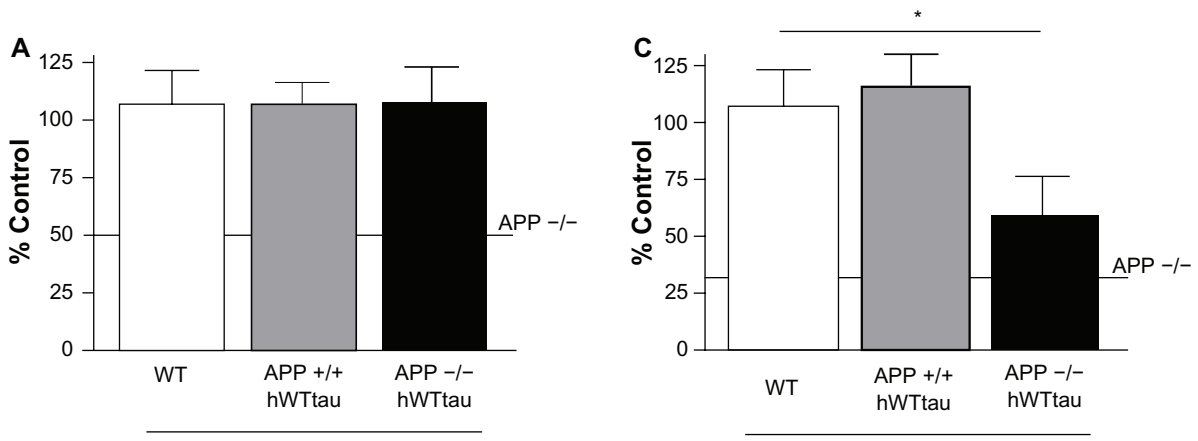
Next, the results from MEMRI measurements from APP +/+ hWTtau and APP –/– hWTtau were acquired and axonal transport rates determined at 2–4 months of age and 12–17 months of age. Axonal transport rates of APP +/+ hWTtau mice at 2–4 months of age are equivalent to rates acquired from WT control animals (Fig. 2A, B), indicating that overexpression of human wildtype tau does not affect axonal transport rates. Importantly, axonal transport rates of the APP –/– hWTtau mice are also equivalent to the age-matched WT control animals indicating that increased levels of normal human tau can overcome axonal transport rate deficits in young APP –/– mice (Fig. 2A, B).

In the aged group, we found that the APP+/+hWTtau mice axonal transport rates remain equivalent to the rates in the age-matched WT control animals (Fig. 2C, D). At the late age point the APP –/– hWTtau miceexhibited a trend towards decreased axonal transport compared to WT controls that did not quite reach significance, ($P < 0.081$) (Fig. 2C). The APP –/– hWTtau axonal transport rates decreased almost 50% compared with WT controls (Fig. 2C) whereas APP –/– decreased 70% compared to WT controls (Fig. 1F). Although the decrease did not reach significance, the aged group of animals demonstrated that the overexpression of human wildtype tau offers a transient attenuation of the axonal transport deficits that occur in the APP –/–.

In the third model tested, we found that APP +/+ R406W mice begin to exhibit axonal transport decreases of 30% at 2–4 months of age compared to WT controls ($P = 0.1486$) (Fig. 3A, B). The axonal transport measurements of the combined genotype APP –/– R406Wtau mice compared to WT were significantly reduced at 2–4 months of age (**$P < 0.0039$) representing a 75% percent decrease (Fig. 3A, B). This decrease is approximately 25% more than the APP –/– versus WT (Fig. 1E). These data reveal that loss of APP function compounds the effects of mutant tau early on axonal transport. Axonal transport deficits also occur in the aged APP +/+ R406Wtau mice and APP –/– R406Wtau mice (Fig. 3C, D). At 12–17 months of age APP +/+ R406Wtau mice (**$P = 0.0014$) exhibited significantly impaired axonal transport, as did APP –/– R406Wtau mice (*$P = 0.0430$) compared to WT controls (Fig. 3C, D).

Discussion

First, our data demonstrate that axonal transport decreases significantly in young APP –/– mice and that this deficit persists in the aged animals, asserting that APP is requisite for normal axonal transport. These results corroborate previous reports in the first histology of APPL –/– *D. melanogaster*[11] presented with axonal swellings which are indicative of axonal transport deficits, and in the second



B

Genotype	Δ in SI/Time
WT	0.01303 ± 0.001843 N = 10
APP +/+ hWTtau	0.01330 ± 0.002951 N = 7
APP −/− hWTtau	0.01317 ± 0.002041 N = 7

D

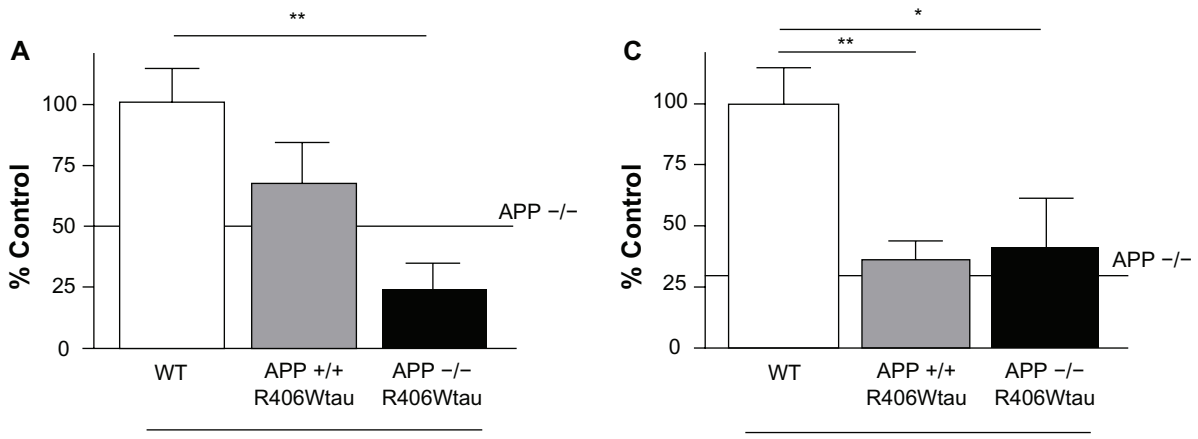
Genotype	Δ in SI/Time
WT	0.01194 ± 0.001766 N = 13
APP +/+ hWTtau	0.01329 ± 0.002092 N = 6
APP −/− hWTtau	0.006374 ± 0.00224 N = 8

Figure 2. APP +/+ hWTtau overexpression overcomes the APP –/– axonal transport deficits at an early age point. **A, C**) Axonal transport reported as percent of control. **A**) 2–4 mos axonal transport in the APP +/+ hWTtau is equivalent to WT. The combined genotype of APP –/– hWTtau also has axonal transport equivalent to those of WT. (ANOVA $P = 0.9941$). **C**) 12+ mos axonal transport indicate that with age the ability of APP +/+ hWTtau overexpression to improve the APP –/– axonal transport deficit is minimized. Axonal transport of the APP +/+ hWTtau group is equivalent to WT control. APP –/– hWTtau group shows a ~50% decrease in axonal transport indicating that deficits in axonal transport do present with aging with the combined genotype. (ANOVA $P = 0.0810$). **B, D**) Raw axonal transport values ± SEM.

the C-terminus of APP sufficiently medieated fast axonal transport in squid axoplasm.[28] The decreased axonal transport in the APP –/– mice also corroborate another study of the same APP –/– mouse, which indicated that the levels of cargo proteins were reduced in the proximal ligation end of sciatic nerves, although global protein levels remained comparable to controls.[9] The APP –/– deficits in axonal transport reported here combined with the previous studies emphasize the importance of APP on functional axonal transport.

This current demonstration of axonal transport deficits in the absence of APP conflicts with a previous report.[10] Potential explanation for the discrepancy includes the global effect measured here compared to the transport of specific proteins of interest measured in the previous report, kinesin-1 and tyrosine kinase receptors. Additionally, timing of the transport measurement can affect the results as we showed changes prior to 2 hours and while the conflicting study reported no changes in transport 24 hours following neuronal ligation.[10] Manganese transport deficits in kinesin-1 mutants, documented to have axonal deficits,[29] are not observed to have differences in transport compared to controls at 24 hours where as they do at 2.5 hours indicating that fast axonal transport deficits need to be tested for at timepoints prior to 24 hours.[30]

We then tested whether increased levels of normal human wildtype tau could modulate the effect of APP on axonal transport *in vivo*. The control group of human wildtype tau overexpression resulted in normal axonal transport at young and aged time points, indicating that overexpression of normal tau in the absence of APP abnormalities bears no consequence on the axonal transport. When combined with APP –/–, the APP –/– hWTtau mice showed axonal transport that were indistinguishable from WT in young animals in contrast to APP –/– alone indicating amelioration of the deficit by hWTtau. Previous reports on the same tau model used in this study showed that the deficits that did develop—reduced spatial learning, synaptic loss, and reduced neuronal activity—occurred later in adulthood (20–24 months).[24] Specifically, Kimura and colleagues reported that tau phosphorylation did not increase prior to 8 months of age, but by 20–24 months age observation of hyperposphorylated tau occurred in concomitant fashion with the other neuronal deficits. We found that the hWTtau model had normal axonal transport by 17 months of age, suggesting that perhaps tau hyperphosphorylation had not yet occurred.



B

Genotype	Δ in SI/ Time
WT	0.01303 ± 0.001843 N = 10
APP +/+ R406Wtau	0.008786 ± 0.002115 N = 5
APP −/− R406Wtau	0.003143 ± 0.00139 N = 5

D

Genotype	Δ in SI/ Time
WT	0.01194 ± 0.001766 N = 13
APP +/+ R406Wtau	0.00434 ± 0.0008501 N = 11
APP −/− R406Wtau	0.004884 ± 0.002333 N = 5

Figure 3. Axonal transport decreases dramatically when mutant tau is combined with APP –/–. **A**) APP +/+ R406Waxonal transport begins to decrease at 2–4 mos. The combined genotype APP –/– R406Wtau has a 75% decrease in axonal transport compared to WT. (ANOVA **P* = 0.0120; post t-tests: WT vs. APP +/+ R406Wtau *P* = 0.1486; WT vs. APP –/– R406Wtau ***P* = 0.0039). **C**) Aging significantly decreases APP +/+ R406Waxonal transport by 12+ mos of age compared to WT. The APP –/– R406Wtau group continues to exhibit significantly reduced axonal transport in comparison to WT. (ANOVA ***P* = 0.0024; post t-tests: WT vs. APP +/+ R406Wtau ***P* = 0.0014; WT vs. APP –/– R406Wtau **P* = 0.0430). **B, D**) Raw axonal transport values ± SEM. **Note:** *indicates significant difference in means.

However, the combined genotype of APP –/– hWTtau did begin to show deficits in axonal transport with aging, indicating that the hWTtau suppression of the APP –/– phenotype diminishes with age. These data indicate that increased levels of human wildtype tau compensated for the loss of APP in young animals. It remains to be determined whether restoration in axonal transport is related to changes in tau phosphorylation.

In the APP +/+ R406Wmouse model, axonal transport declined in young animals, the effect of which was compounded by the loss of APP in the APP –/– R406Wtau mouse model. Previously, the APP +/+ R406W mice were reported to develop accumulations of neuronal filaments, memory deficits and microtubule disruption.[23] The particular model used here expresses mutated tau driven by an αCAMKII promoter to induce accumulation of mutant tau in the forebrain regions postnatally, including the olfactory bulb.[23] This accounts for the slight decrease in axonal transport of the APP +/+ R406Wgroup at 4 months of age. The axonal transport deficits in the APP –/– R406Wtau group are clearly visible by 4 months of age and represent a combined deleterious affect on axonal transport. The effect of the APP –/– R406W combined genotype suggests a role for both APP and tau in the axon, potentially on microtubule stabilization. Whether these data are the result of hyperphosphorylation of tau is unclear because *in vitro* work with the R406W mutation has led to mixed results as to whether tau is hyperphosphorylated or hypophosphorylated depending upon the particular cell line used.[23,31–33] Samples from human tissue of patients identified with the tauopathy FTDP-17 induced by the R406W mutation do exhibit tau hyperphosphorylation,[34] indicating further measurements are needed to establish whether this occurs in mouse models of the disease. The decreases in the axonal transport rate of the APP +/+ R406W model in combination with the loss of APP further illustrate a role for APP in axonal transport function in conjunction with tau.

In summary, we focused on the normal function of APP and how it interacts with normal and mutated forms of human tau in order to further understand the role of APP on axonal transport. Our results indicate that an interaction of tau and APP occurs normally and that changes to APP levels can affect the balance of this relationship and contribute to disease processes. Utilizing MEMRI we were able to identify a neuronal deficit indicating that axonal transport

isaffected earlier than other pathological indicators. The data reported here support the axon as a likely region of interaction between tau and APP because both proteins normally affect microtubule stability and axonal transport. Further characterization of this combined effect on microtubule stability and axonal transport could lead to identification of potential methods for therapeutic intervention of AD.

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Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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