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# Elevated Aβ and Apolipoprotein E in AβPP Transgenic Mice and Its Relationship to Amyloid Accumulation in Alzheimer's Disease

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## Abstract

**Background:** Amyloid-$\beta$ (A$\beta$) accumulates in plaques and as cerebral amyloid angiopathy (CAA) in the brains of both Alzheimer's disease (AD) patients and transgenic A$\beta$PPswe/tg2576 (tg2576) mice. Increasingly, evidence in humans and mice shows this process to be modulated by apolipoprotein E (apoE).
**Materia fls and Methods:** To explore this relationship, we measured apoE and A$\beta$ levels in brains of tg2576 mice and controls at intervals between 2 and 20 months. In addition, A$\beta$ concentrations in plasma and muscle of these animals were also quantified.
**Results:** Quite strikingly, we found that the amount of tg2576 mice brain apoE was elevated by an average of 45%, relative to the control mice from 2 months on. The level of brain apoE soared after 14 months to almost 60% greater than the level found in control mice. A$\beta$ concentrations in brains before 9 months were less than 2 ng/mg of protein, but by 14 months concentrations rose to 8.7 ng/mg, and by 20 months to 47 ng/mg. In plasma, we noted that the levels of A$\beta$ in tg2576 mice declined from above 30 ng/ml prior to 12 months to 14 ng/ml by 14 months. Histology showed that A$\beta$ plaques and CAA began to be discernible in the tg2576 mice at about 9 and 20 months of age, respectively.
**Conclusions:** ApoE was immunocytochemically detected in neuritic plaques that were positive for thioflavine-S. We suggest that the elevation of brain apoE in tg2576 mice participates in an age-related dysregulation of A$\beta$ clearance and signals the start of A$\beta$ sequestration during the time of cognitive dysfunction.

## Introduction

Alzheimer's disease (AD) is characterized by progressive dementia and by pathological fibrillar deposits in the brain. Neurofibrillary tangles composed of hyperphosphorylated tau and glycolipids accumulate within neurons (1,2). Amyloid-$\beta$ (A$\beta$) peptides, primarily as A$\beta$1-40 and A$\beta$1-42, accumulate in fibrillar and nonfibrillar forms as senile plaques in the extracellular spaces of the brain parenchyma and in the arterial walls, both within the brain and in the leptomeninges as cerebral amyloid angiopathy (CAA; 3–6). The contribution of cerebral vasculature pathology to the dementia of AD in relation to CAA, atherosclerosis and arteriosclerosis, and embolic-ischemic brain damage is increasingly recognized (7).

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Amyloid-$\beta$, a natural product of A$\beta$ precursor protein (A$\beta$PP) metabolism, begins to accumulate in the brain at around 50 years of age and shows a very substantial rise in patients with AD (8). The molecular basis for this A$\beta$ sequestration and deposition remains obscure. However, recent studies implicate apolipoprotein E (apoE) in this process. ApoE is a protein involved in the transport of cholesterol and triglycerides (9). In humans, there are three isoforms of apoE (apoE2, E3 and E4), which differ by only one or two amino acids. Numerous studies show that apoE4 status increases the risk for AD or accelerates its age of onset (10,11). ApoE4 dose-dependency increases the levels of A$\beta$ peptide in aging and AD brains, in both parenchyma and the cerebral vasculature (12,13).

In light of the above findings, we sought to determine the status of apoE levels in the brains of tg2576 A$\beta$PP mice, in conjunction with those levels of A$\beta$ found centrally in the brain and peripherally in the plasma. In addition, the levels of cholesterol, $\alpha$1-antichymotrypsin, $\alpha$-synuclein and C1q in the brains and A$\beta$ in muscle of tg2576 mice and non-tg mice were measured.

## Materials and Methods

*Transgenic Mice*

The transgenic A$\beta$PPswe mice from the 2576 line (C57B6/SJL) used in the present study were developed by Hsiao et al. (14). These mice contain the human A$\beta$PP695, with the double mutation Lys670→Asn, Met671→Leu (A$\beta$PP$_{770}$ numbering), which was inserted into a hamster prion protein (PrP) cosmid vector. The mice brains used in the present study were provided by M. Mullan Roskamp Institute, University of South Florida, Tampa FL. Previous histopathological analysis showed that between the young (2–8 months) and the old (11–13 months) tg2576 mice there was a 14-fold increase in the amount of A$\beta$42 in the brain and a 5-fold increase in A$\beta$40 (14). The tg2576 mice exhibited learning and memory impairment at 9–10 months of age.

*Tissue Preparation, Chromatography, and A$\beta$ Quantitation*

A total of 18 tg2576 and 18 control mice were involved in this study. The mice were terminated at 2, 4, 9, 12, 14, and 20 months of age. In both groups, each age was represented by three mice. Blood was immediately collected by venipuncture from the brachiocephalic vein using a 1 ml syringe primed with 30 $\mu$l of $K_3$EDTA (4.5 mg) and centrifuged to separate the plasma from the cells. The right hemisphere, pectoralis major and sternomastoid muscles were removed and immediately frozen at $-70$°C until the moment of processing. For immunostaining, the left hemispheres were fixed in 4% paraformaldehyde (Fluka Chemie AG, Bucks, Switzerland) for 48 hr and stored in phosphate-buffered saline (PBS) at 4°C.

The right cerebral cortex (120–180 mg) was homogenized in 5 volumes of 20 mM Tris-HCl (Sigma Chemical Company, St. Louis, MO), pH 7.5, and a sample of 5 $\mu$l was diluted 1000 fold to measure total protein concentration using the Micro BCA protein assay kit (Pierce, Rockford, IL). 50 $\mu$l of the brain homogenate was set aside for cholesterol measurements. The remaining homogenate was immediately centrifuged at 500,000 $\times$ g for 15 min at 5°C. The supernatant was used for the determination of soluble A$\beta$. For the determination of insoluble A$\beta$, the pellet was homogenized in 1 ml of 98% glass-distilled formic acid (GDFA) and centrifuged at 500,000 $\times$ g for 20 min at 5°C. From each mouse, 200 mg of skeletal muscle was finely minced, directly homogenized in 5 ml of GDFA and centrifuged at 200,000 $\times$ g for 30 min at 5°C. 500 $\mu$l harvested from the middle volume of the clear acid supernatant, from either brain or muscle, was loaded onto a Superose 12 column (HR 10 $\times$ 300 mm, Amersham-Pharmacia, Piscataway, NJ). To separate the A$\beta$ peptides from other circulating molecules, 50 $\mu$l of plasma was diluted with 450 $\mu$l of 98% GDFA and applied onto the Superose 12 column. All chromatographic separations were carried out using a Amersham-Pharmacia fast performance liquid chromatography (FPLC) apparatus, developed in 80% GDFA, at a flow rate of 15 ml/hour at room temperature, and monitored at 280 nm. The chromatographic fractions corresponding to the 4.0–4.5 kDa (2 ml) were pooled and, after addition of 20 $\mu$l of 10% betaine (Sigma Chemical Co.), the acid was totally removed by vacuum centrifugation.

FPLC separated 4.0 to 4.5 kDa A$\beta$-containing-fractions from plasma, brain, and muscle. A$\beta$ levels in these fractions were determined using the europium immunoassay (EuIA) (Wallac Inc., Gaithersburg, MD) as described

in detail elsewhere (15). Polyclonal antibodies, R163 and R165 (provided by P. Mehta, New York Institute for Basic Research and Mental Disabilities, Staten Island, NY), raised against the C-terminal sequence of A$\beta$ residues 34–40 and 36–42, respectively (16), were used as capture antibodies. The monoclonal antibody, 4G8 (Senetec, Maryland Heights, MO), against A$\beta$ sequence residues 17–24, was labeled with europium (Eu) and used as a reporter antibody. In an independent experiment, the monoclonal antibody 6E10 (Senetec), against A$\beta$ residues 1–16, was used as a capture antibody. To confirm the reliability of our data, a second set of polyclonal antibodies, R209 and R226, raised against A$\beta$ residues 34–40 and 36–42, respectively, kindly provided by P. Mehta was also used as capture antibodies.

*Western Blot Analysis and Protein Quantification*

Western blot analysis was used to determine protein levels in the brain. For the detection of A$\beta$PP, monoclonal antibody 22C11 (Boehringer-Mannheim, Indianapolis, IN) was used as the primary antibody. For apoE, we used a goat anti-rat polyclonal antibody, kindly provided by C. L. Bisgaier (Esperion Therapeutics, Ann Arbor, MI) and P. J. Dolphin (Dalhousie University, Halifax, NS, Canada). The antibody was raised against the rat apoE that was purified by the method of Holmquist and Carlson (17) and its purity was assessed by SDS-PAGE (18). The specificity of this antibody was verified by two-dimensional crossed immunoelectrophoresis (19). For C1q and $\alpha$1-antichymotrypsin, the antisera were obtained from Dako (Glostrup, Denmark). For $\alpha$-synuclein quantitation, the antibody was kindly donated by Eliezer Masliah (University of California at San Diego, San Diego, CA). All Western blots were developed with the Supersignal Chemiluminescent Substrates (Pierce) and the relative intensities of the bands were measured on a scanning ChemiImager (Alpha Innotech Corp., San Leandro, CA).

*Brain Cholesterol Quantitation*

Cholesterol levels in brain were estimated using the modified Folch technique for lipid extraction (20). Briefly, to each 50 $\mu$l of initial brain homogenate, 10 $\mu$g of 4-cholesten-3-one (ICN Biomedicals Inc., Aurora, OH) was added as an internal standard for high performance liquid chromatography (HPLC). The sample was homogenized in 250 $\mu$l of chloroform:methanol (2:1, v/v) and filtered through Whatman No. 1 filter paper. Another 250 $\mu$l of chloroform:methanol mixture were used to re-extract the residue. Water (100 $\mu$l) was added to the filtrates and the organic layer was collected after centrifugation at 12,000 $\times$ g for 10 min. The lipid extracts were completely dried under a fine nitrogen stream and then dissolved in 500 $\mu$l of 5% 2-propanol:hexane (volume per volume; v/v). The cholesterol was purified by HPLC (Thermo Separation Products, Freemont, CA) on a silica normal phase column (Zorbax SIL, 4.6 $\times$ 250 mm, 5 $\mu$m) at a flow rate of 1 ml per min. The relative absorbance values at 208 nm between the internal standard and the cholesterol were considered in the final calculation of brain cholesterol.

*Immunocytochemistry*

The paraformaldehyde-fixed left hemisphere was sectioned at 40 $\mu$m and stained with antibodies 4G8 and 6E10 (Senetec) for A$\beta$ or anti-apoE antibody (19). Sections were incubated in primary antibodies at 1:1000 overnight at 4°C, followed by a biotinylated secondary antibody (1:2000) and an avidin-biotin-peroxidase (ABC, Vector Laboratories, Burlingame, CA) using 3,3′-diaminobenzidine as the substrate. Control sections were treated identically, except for the omission or preabsorption of the primary antibody with 100 $\mu$g/ml synthetic A$\beta$ 1–40. A section of frontal cortex from a AD case was used as a positive control.

*Statistical Analyses*

To study the within-group relationships, regression models (linear or nonlinear) were separately applied to the tg2576 mice or non-tg mice. When group and age were incorporated as independent variables, a 2-way factorial analysis of variance (ANOVA) was used. Post-hoc multiple comparison (Fisher's LSD) was employed to analyze individual significant effects.

## Results

*Apolipoprotein E Levels in Brain*

The levels of apolipoprotein E in the brain of tg2576 mice were higher than those observed in the non-tg mice ($F = 24.63$; $p < 0.001$). In addition, in the former, there was a steady increase in apoE with age (Fig. 1A). During the first 12 months, this elevation was relatively

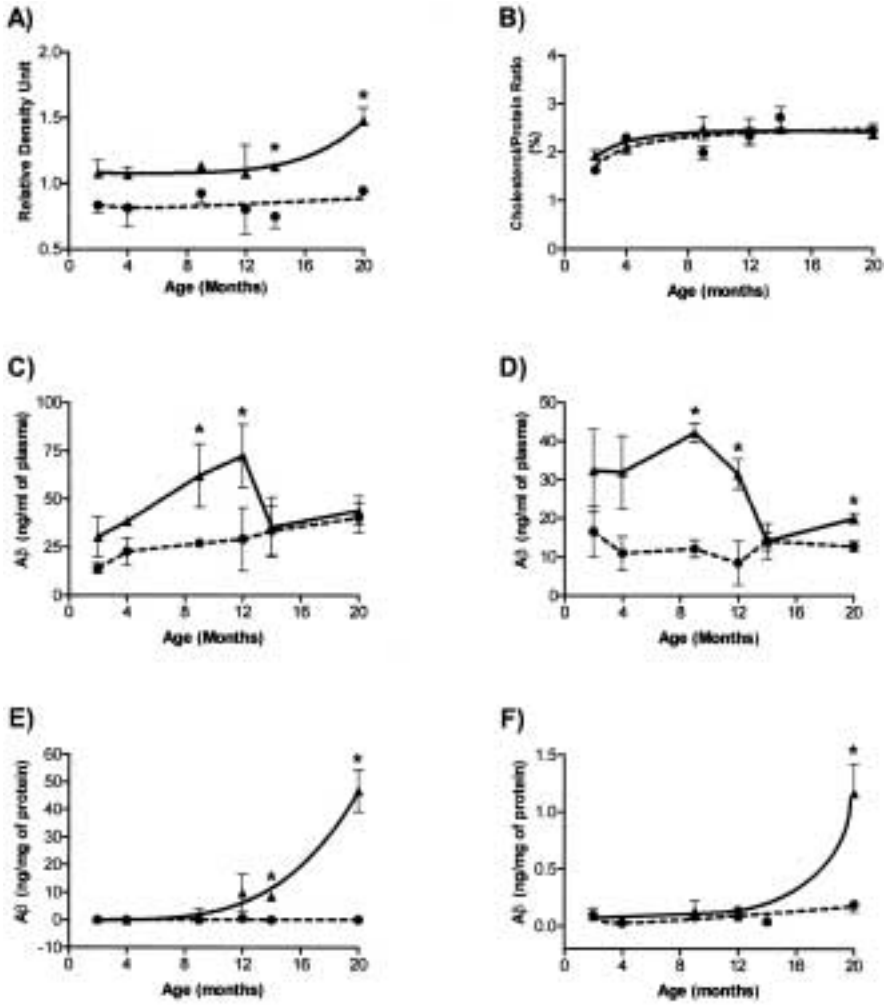


**Fig. 1. Quantification of apolipoprotein E (apoE) and cholesterol in brain and of amyloid-β (Aβ) peptides in plasma and brain.** Concentrations for tg2576 (solid line) mice and control (hyphened line) mice are shown at different ages. (A) The levels of apoE were already elevated in the tg2576 mice at 2 months of age, relative to the controls. This elevation remained constant up to 12 months of age and significantly thereafter (*, $p < 0.05$). (B) The brain total cholesterol levels, isolated and quantified by high performance liquid chromatography (HPLC) were essentially the same in both the tg2576 and control mice at all ages. (C) The levels of plasma A$\beta$ peptides detected by antibody R163 (A$\beta$N-40) and antibody R165 (A$\beta$N-42). (D) The levels of plasma A$\beta$ peptides, quantified by antibody 6E10 (A$\beta$1-40/42). In both instances (C and D), the total plasma A$\beta$ was significantly increased (*, $p < 0.05$) in the tg2576 mice at the ages of 9 and 12 months, relative to controls. (E) and (F) The amounts of water-insoluble and water-soluble A$\beta$ peptides, respectively, in the tg2576 and control mice brains. After 12 months of age, the quantities of total A$\beta$ in the tg2576 mice brain increased in a quasi-exponential mode.

constant in the tg2576 mice, with levels 30–34% greater than those in the non-tg mice. However, this difference became more pronounced at 14 and 20 months of age (53% and 57%, respectively), reaching statistical significance at these ages.

Since apoE is a cholesterol carrier, we then sought to observe the changes of brain cholesterol. Our results demonstrated that the increase in brain apoE levels was not a reflection of increased brain cholesterol. The levels of cholesterol in the brains of tg2576 and non-tg mice progressively increased with age ($F = 5.73$; $p = 0.001$), but no statistical difference was found between the two groups ($F = 0.42$; $p > 0.5$; Fig. 1B).

As expected, the A$\beta$PP values were almost ten times higher in the brains of tg2576 mice (Mean relative optical density (OD) units = 16.9, range: 14–20) than in the non-tg mice

(Mean = 1.7, range: 0.27–4.5). However, these levels of AβPP remained stable throughout the life of the tg2576 animals (Fig. 2). The elevation of apoE was unique among the other brain proteins that we studied. No statistically significant differences were found in the amounts of C1q, α1-antichymotrypsin and α-synuclein in the brains of tg2576 mice and non-tg mice at all ages (data not shown).

*Aβ Levels in Plasma*

There was a significant increase with age in the levels of total Aβ, defined as the sum of Aβ40 and Aβ42, in the plasma of the tg2576 mice from 2 months to 12 months ($r = 0.678$, $p = 0.022$; Fig. 1C). As age advanced, tg2576 mice showed a sharp decrease in the plasma Aβ levels at 14 months ($p < 0.05$) followed by a moderate increase by 20 months. During this period, the Aβ42 was, on the average, about three times more abundant than Aβ40 in the plasma of tg2576 mice. Unlike the tg mice, the Aβ levels in the plasma of the non-tg littermates increased slowly ($r = 0.507$; $p = 0.032$) throughout the entire experimental period (Fig. 1C). In the non-tg mice, the overall average ratio of Aβ42 over Aβ40 was 2:1. A similar time-dependent trend was observed in the tg2576 mice when the 6E10 and 4G8 antibodies were used to measure only the human form of total Aβ in plasma (Fig. 1D). As expected, the non-tg mice demonstrated basal levels of Aβ in plasma with the 6E10 antibody, since it did not recognize the sequence of amino acids 1-16 of the mouse Aβ peptide (Fig. 1D).

In contrast to plasma, there was no statistically significant difference between the levels of Aβ in skeletal muscle of tg2576 and non-tg mice at the ages investigated ($f = 0.755$; $p > 0.5$). The average value for total Aβ in the tg2576 mice was 65 ng/g of muscle (range: 31–104 ng/g of muscle) and for the non-tg mice was 100 ng/g of muscle (range: 19–178 ng/g).

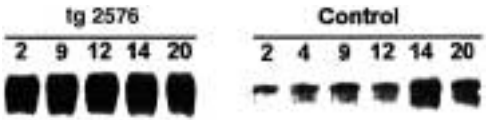


**Fig. 2. Expession levels of amyloid-β precursor protein in tg2576 and control mice.** Expression levels are shown at different ages, detected by Western blots using the 22C11 antibody.

*Aβ Levels in Brain*

The drop in plasma Aβ levels in tg2576 mice was inversely correlated with an exponential increase in the brain Aβ levels. Until 9 months of age, the levels of soluble and insoluble Aβ40 and Aβ42 in the brain remained below 2 ng/mg of protein in both the tg2576 and non-tg mice (Fig. 1E and 1F). By 14 months of age, the total Aβ in the tg2576 mice had reached 8.7 ng/mg of protein and, by 20 months, it was 47 ng/mg of protein (Fig. 1E). In the brains of the 20-month-old tg2576 mice, Aβ42 was five times more abundant than Aβ40. Non-tg mice showed no substantial change in the levels of Aβ in the brain with age, remaining below 0.7 ng/mg of protein from 2 to 20 months of age (Fig. 1E).

Interestingly, when the amounts of total brain Aβ were plotted against the levels of brain apoE in the tg2576 mice, a significant correlation was found ($r^2 = 0.545$; $p = 0.006$). However, since the amounts of total brain Aβ in tg2576 mice displayed a second-order hyperbolic distribution (Fig. 1E and 1F), the log values of the total brain Aβ levels were plotted against apoE concentrations. Using this approach, a positive correlation was also demonstrated ($r^2 = 0.349$; $p = 0.043$).

*Neuropathology*

At all ages studied, there was a total absence of amyloid deposits in the non-tg mice. In contrast, in the tg2576 mice, sparse diffuse plaques and few small punctuated amyloid cores positive for thioflavine S were evident at 12 months of age in both the hippocampus and the cortex. By 14 months, the number of star-like amyloid plaques stained by thioflavine S and Campbell-Switzer histochemistry, as well as diffuse amyloid deposits, were numerous in both regions of the brain. In the 20-month-old tg2576 mice, there were abundant and larger amyloid plaques with dense cores, which in many instances, closely assembled and occasionally coalesced (Fig. 3A and B). Also at 20 months of age, cerebrovascular amyloid deposits were visible for the first time in larger vessels, especially in the hippocampal fissure. Amyloid plaques positive for thioflavine S were also intensely stained for apoE immunocytochemistry (Fig. 3C).

## Discussion

Studies in AβPP tg2576 mice suggested that apoE may play a paradoxical role in the clearance of Aβ from brain. Decreased Aβ levels

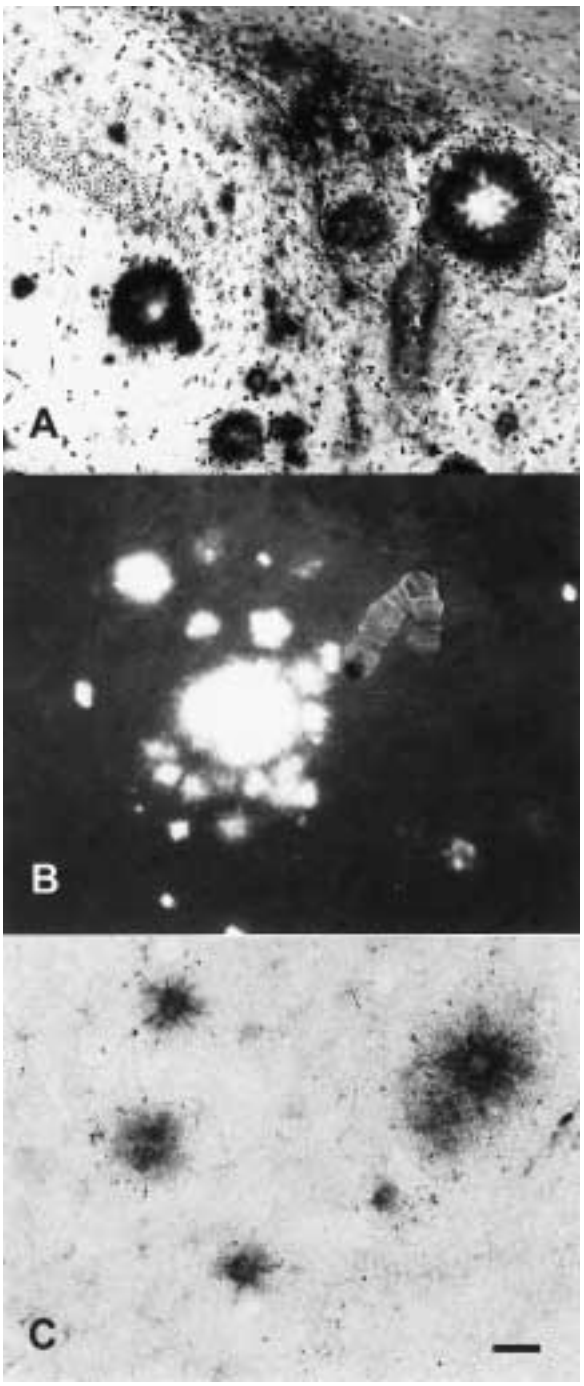


**Fig. 3. Amyloid deposits in tg2576 mice.** (A) Campbell-Switzer silver stain. (B) Thioflavine-S stain for amyloid. (C) Immunochemistry with polyclonal antibody against rodent apolipoprotein E. All three views were from the tg2576 mice at 20 months of age. No cerebrovascular amyloid deposits were observed prior to 20 months of age. The amyloid deposits range in size from 10 $\mu$m to 110 $\mu$m. Bar = 40 $\mu$m.

caused by maintaining A$\beta$PP mice on a high cholesterol diet required the presence of apoE (21). In contrast, Bales and colleagues (22,23) showed that the deposition, not the clearance, of A$\beta$ in the brains of aging PDAPP tg mice was apoE dependent. In an effort to reconcile these seemingly contradictory roles, we followed the natural history of A$\beta$ and apoE levels in the brains of aging A$\beta$PP tg2576 mice. To our surprise, the levels of apoE and A$\beta$ increased in parallel with age, at the expense of decreasing A$\beta$ levels in plasma from these animals. This finding implied reduced clearance of the peptide from the brain. The elevation of apoE was unique among the brain proteins that we studied. Moreover, the change in apoE occurred without a significant change in brain cholesterol. Thus, our results favor a role for apoE in the sequestration of A$\beta$ in the brains of aging tg2576 mice.

Considerable importance has been attached to apoE status in AD, since individuals who are apoE4 are at increased risk for this dementia (24,25). However, the mechanism or mechanisms responsible for the apoE4 effect in AD must be elucidated. In light of our present results, it is provocative to note that human apoE4 is analogous to the apoE found in mice (26). A consistent consequence of carrying the apoE $\epsilon$4 gene is an increased amyloid burden in brain and cerebral vasculature. In AD, apoE4 is linked to increased numbers of amyloid plaques in brain parenchyma, as well as more abundant CAA (12,13). A cross-sectional study of over 200 brains from nondemented elderly subjects reveals that individuals bearing the gene for apoE4 have increased diffuse deposits of A$\beta$42, in addition to a greater incidence of CAA (27). Parenthetically, these diffuse deposits are common in subjects with mild cognitive impairment (MCI), a condition considered prodromal to the clinical onset of AD (28,29). A similar association between apoE4 status and amyloid deposition also was seen after traumatic brain injury resulting from accidents (30) or boxing (31). The importance of apoE4 to amyloid deposition was underscored by the work of Carter et al. (32) who showed that tg2576 mice bearing human apoE4 produced more amyloid plaques than those mice without apoE or with human apoE3 (32). This latter result, in particular, supports the notion that apoE4 participates directly in the deposition of A$\beta$ in brain

Numerous studies have examined the possible A$\beta$ and apoE interactions responsible for

the apolipoprotein-associated changes in AD. In general, these studies show that the interactions of Aβ with apoE are isoform-dependent when the apolipoprotein is lipidated. Several laboratories report that human apoE4 in a lipid complex binds less well to Aβ than either apoE3 and apoE2 (33–35). Genetically substituting cysteine for arginine at position 158 is sufficient to make apoE4 behave like apoE3, with respect to Aβ binding (36). Cell culture studies report conflicting effects of apoE4 on the clearance of Aβ. ApoE4 stimulates the uptake of Aβ by primary cultures of rat hippocampal neurons (37), but inhibits Aβ uptake by Chinese hamster ovary cells (38).

A number of facets of apoE4's functions or properties might lead to increased Aβ in the central nervous system (CNS). Some, but not all, studies indicate that apoE4 increases the fibrillogenesis of Aβ, thereby, promoting its deposition (39–42). The relative inability of apoE4 to bind Aβ compared with the other apoE isoforms (33,36) might also hinder Aβ clearance (38,43). The overexpression of transforming growth factor-β (TGF-β) in brain, which increases apoE expression along with that of heparan sulfate proteoglycan (HSPG), promotes amyloid deposition in the cerebral vasculature of AβPP tg mice (44). Thus, it is also possible that apoE4 provides an essential component for the development of an extracellular milieu favoring Aβ deposition.

It is well recognized that apoE binds Aβ and acts as a transporter for this molecule in both the CNS and in the circulation (43,45,46). Lipidated apoE also interacts with HSPG prior to endocytosis by the low density lipoprotein (LDL) receptor and LDL receptor-related protein (LRP; 47). Chemical and immunocytochemical analyses have demonstrated the presence of HSPG, apoE and Aβ peptide in both neuritic plaques and CAA (48,49). When HSPG levels are experimentally up-regulated, Aβ is profusely deposited in the vascular walls (44). An even more intriguing observation is that the presence of apoE increases the sulfation of glycosaminoglycans (GAG; 50). Furthermore, the moieties of GAG are relevant to Aβ aggregation, since they can enhance the rate of fibrillogenesis (51). These data suggest that apoE in AD has significant pleiotropic effects: (1) as an Aβ carrier molecule, the expression of apoE responds to Aβ concentrations and is upregulated when the Aβ levels increase in the brain; (2) elevations in apoE may promote sharply increased GAG sulfation; and (3) because both apoE and Aβ have a high affinity for GAG, the enhanced GAG sulfation may promote Aβ fibrillogenesis. Future experiments will resolve which of the possible mechanistic roles of apoE is critical to the deposition of Aβ in the brains of AβPP transgenic mice.

It is remarkable that the observed rise of Aβ in the plasma peaks at a time coinciding with cognitive decline in tg2576 mice. Although some learning impairments can be detected in the tg2576 mice as early as 3 months of age, it is only after 9 months that impairment of spatial memory, measured by the Morris water maze test, reaches statistical significance (14,52). Oddly, a somewhat similar pattern is observed in people with MCI. Mayeux and his colleagues (53) reported recently that MCI patients had higher plasma levels of Aβ than normal controls. The elevated levels of plasma Aβ are associated with an increased risk for AD. Yet, people with MCI fail to manifest the robust histopathological hallmarks, plaques and tangles, found in AD brains (28). Instead, MCI individuals manifest diffuse plaques (28,29) and some hippocampal atrophy (54), in conjunction with their cognitive decline. Interestingly, the presence of plaques and hippocampal atrophy in nondemented or cognitively impaired individuals is often associated with cardiovascular disease (55,56).

One must wonder if the rise in plasma Aβ signals the beginning of a neurodegenerative process that ultimately leads to AD. Based on our current results in tg2576 mice, it is conceivable that plasma Aβ is derived from the brain and transported into the periphery. Failure to maintain this clearance could result in the accumulation of Aβ in the form of fibrillar amyloid plaques. However, implicit in this conjecture is the possibility that it is the pathology leading to the failure to clear Aβ, rather than the accumulation of Aβ itself, that is responsible for the pathological and behavioral changes of AD. From this perspective, the AβPP transgenic mice, although not recapitulating the full spectrum of AD neuropathology, might reflect both central and peripheral changes indicative of the incipient stages of the dementia. If true, then the AβPP tg mice may be invaluable for elucidating the biochemical, physiological, and morphological underpinnings of Alzheimer dementia *in statu nascendi*. Thus, detailed molecular characterization of these murine models might provide indica-

tions of the optimal therapeutic prevention of age-related dementia. In light of our results, it will be of particular interest to determine precisely the role that apoE plays in the clearance or accumulation of A$\beta$ in the brains of aging tg2576 mice.

## Acknowledgments

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## References

1. Lee VM, Balin BJ, Otvos LJ, Trojanowski JQ. (1991) A68: a major subunit of paired helical filaments and derivatized forms of normal Tau. *Science* **251**: 675–678.
2. Goux WJ, Rodriguez S, Sparkman DR. (1995) Analysis of the core components of Alzheimer paired helical filaments. A gas chromatography/mass spectrometry characterization of fatty acids, carbohydrates and long-chain bases. *FEBS Lett.* **366**: 81–85.
3. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **82**: 4245–4249.
4. Roher AE, Lowenson JD, Clarke S, et al. (1993) Structural alterations in the peptide backbone of $\beta$-amyloid core protein may account for its deposition and stability in Alzheimer's disease. *J. Biol. Chem.* **268**: 3072–3083.
5. Roher AE, Lowenson JD, Clarke S, et al. (1993) $\beta$-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **90**: 10836–10840.
6. Alonzo NC, Hyman BT, Rebeck GW, Greenberg SM. (1998) Progression of cerebral amyloid angiopathy: accumulation of amyloid- $\beta$40 in affected vessels. *J. Neuropathol. Exp. Neurol.* **57**: 353–359.
7. Emmerling MR, Gracon S, Roher AE. (1999) Towards a unifying hypothesis of Alzheimer's disease pathogenesis and pathophysiology. In: Gauthier S (ed.) *Clinical Diagnosis and Management of Alzheimer's Disease*. Martin Dunitz, London, pp. 33–53.
8. Funato H, Yoshimura M, Kusui K, et al. (1998) Quantitation of amyloid $\beta$-protein (A$\beta$) in the cortex during aging and in Alzheimer's disease. *Am. J. Pathol.* **152**: 1633–1640.
9. Weisgraber KH, Mahley RW. (1996) Human apolipoprotein E: the Alzheimer's disease connection. *FASEB J.* **10**: 1485–1494.
10. Corder EH, Saunders AM, Strittmatter WJ, et al. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**: 921–923.
11. Meyer MR, Tschanz JT, Norton MC, et al. (1998) APOE genotype predicts when—not whether—one is predisposed to develop Alzheimer disease. *Nat. Genet.* **19**: 321–322.
12. Schmechel DE, Saunders AM, Strittmatter WJ, et al. (1993) Increased amyloid $\beta$-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **90**: 9649–9653.
13. Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, Hyman BT. (1995) Apolipoprotein E$\varepsilon$4 and cerebral hemorrhage associated with amyloid angiopathy. *Ann. Neurol.* **38**: 254–259.
14. Hsiao K, Chapman P, Nilsen S, et al. (1996) Correlative memory deficits, A$\beta$ elevation, and amyloid plaques in transgenic mice. *Science* **274**: 99–102.
15. Kuo YM, Kokjohn TA, Watson MD, et al. (In press) Elevated A$\beta$ 42 in skeletal muscle of Alzheimer disease patients suggest peripheral alterations of APP metabolism. *Am. J. Pathol* **156**: 797–805.
16. Mehta PD, Dalton AJ, Mehta SP, Kim KS, Sersen EA, Wisniewski HM. (1998) Increased plasma amyloid $\beta$ protein 1–42 levels in Down syndrome. *Neurosci. Lett.* **241**: 13–16.
17. Holmquist L, Carlson K. (1977) Selective extraction of human serum very low density apolipoproteins with organic solvents. *Biochim. Biophys. Acta* **493**: 400–409.
18. Dolphin PJ. (1980) Organic extraction and preparative isoelectric focussing of rat serum apolipoproteins. *FEBS Lett.* **117**: 252–258.
19. Dolphin PJ, Wong L, Rubinstein D. (1978) A comparison of some immunological characteristics of very low density lipoproteins of normal and hypercholesterolemic rat sera. *Can. J. Biochem.* **56**: 673–683.
20. Folch J, Lees M, Stanley GHS. (1956) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509.
21. Howland DS, Trusko SP, Savage MJ, et al. (1998) Modulation of secreted $\beta$-amyloid precursor protein and amyloid $\beta$-peptide in brain by cholesterol. *J. Biol. Chem.* **273**: 16576–16582.
22. Bales KR, Verina T, Dodel RC, et al. (1997) Lack of apolipoprotein E dramatically reduces amyloid $\beta$-peptide deposition. *Nat. Genet.* **17**: 263–264.
23. Bales KR, Verina T, Cummins DJ, et al. (1999)

Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 15233–15238.
24. Roses AD. (1996) Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu. Rev. Med.* **47:** 387–400.
25. Poirier J. (1999) Apolipoprotein E4, cholinergic integrity and the pharmacogenetics of Alzheimer's disease. *J. Psychiatry Neurosci.* **24:** 147–153.
26. LaDu MJ, Lukens JR, Reardon CA, Getz GS. (1997) Association of human, rat, and rabbit apolipoprotein E with $\beta$-amyloid. *J. Neurosci. Res.* **49:** 9–18.
27. Walker LC, Pahnke J, Madauss M, et al. (In press) Apolipoprotein E4 promotes the early deposition of A$\beta$42 and then A$\beta$40 in the elderly. *Acta Neuropathol.*
28. Mufson EJ, Chen EY, Cochran EJ, Beckett LA, Bennett DA, Kordower JH. (1999) Entorhinal cortex $\beta$-amyloid load in individuals with mild cognitive impairment. *Exp. Neurol.* **158:** 469–490.
29. Price JL, Morris JC. (1999) Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann. Neurol.* **45:** 358–368.
30. Graham DI, Gentleman SM, Nicoll JA, et al. (1999) Is there a genetic basis for the deposition of $\beta$-amyloid after fatal head injury? *Cell Mol. Neurobiol.* **19:** 19–30.
31. Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, Gandy S. (1997) Apolipoprotein E epsilon 4 associated with chronic traumatic brain injury in boxing. *JAMA* **278:** 136–140.
32. Carter DB, Dunn E, McKinley DD, et al. (1999) Increased levels of apolipoprotein E4 accelerates amyloid deposition in APPsw transgenic mouse brain. *Soc. Neurosci. Abstr.* **25 (part 2):** 1348
33. LaDu MJ, Falduto MT, Manelli AM, Reardon CA, Getz GS, Frail DE. (1994) Isoform-specific binding of apolipoprotein E to $\beta$-amyloid. *J. Biol. Chem.* **269:** 23403–23406.
34. Zhou Z, Smith JD, Greengard P, Gandy S. (1996) Alzheimer amyloid-$\beta$ peptide forms denaturant-resistant complex with type $\epsilon$ 3 but not type $\epsilon$ 4 isoform of native apolipoprotein E. *Mol. Med.* **2:** 175–180.
35. Aleshkov S, Abraham CR, Zannis VI. (1997) Interaction of nascent ApoE2, ApoE3, and ApoE4 isoforms expressed in mammalian cells with amyloid peptide $\beta$ (1–40). Relevance to Alzheimer's disease. *Biochemistry* **36:** 10571–10580.
36. Aleshkov SB, Li X, Lavrentiadou SN, Zannis VI. (1999) Contribution of cysteine 158, the glycosylation site threonine 194, the amino- and carboxy-terminal domains of apolipoprotein E in the binding to amyloid peptide $\beta$ (1–40). *Biochemistry* **38:** 8918–8925.
37. Beffert U, Aumont N, Dea D, Lussier-Cacan S, Davignon J, Poirier J. (1999) Apolipoprotein E isoform-specific reduction of extracellular amyloid in neuronal cultures. *Brain Res. Mol. Brain Res.* **68:** 181–185.
38. Yang DS, Small DH, Seydel U, et al. (1999) Apolipoprotein E promotes the binding and uptake of $\beta$-amyloid into Chinese hamster ovary cells in an isoform-specific manner. *Neuroscience* **90:** 1217–1226.
39. Castano EM, Prelli F, Wisniewski T, et al. (1995) Fibrillogenesis in Alzheimer's disease of amyloid $\beta$ peptides and apolipoprotein E. *Biochem. J.* **306:** 599–604.
40. Evans KC, Berger EP, Cho CG, Weisgraber KH, Lansbury PTJ. (1995) Apolipoprotein E is a kinetic but not a thermodynamic inhibitor of amyloid formation: implications for the pathogenesis and treatment of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 763–767.
41. Sanan DA, Weisgraber KH, Russell SJ, et al. (1994) Apolipoprotein E associates with $\beta$ amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. *J. Clin. Invest.* **94:** 860–869.
42. Wisniewski T, Castano EM, Golabek A, Vogel T, Frangione B. (1994) Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am. J. Pathol.* **145:** 1030–1035.
43. Russo C, Angelini G, Dapino D, et al. (1998) Opposite roles of apolipoprotein E in normal brains and in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **95:** 15598–15602.
44. Wyss-Coray T, Masliah E, Mallory M, et al. (1997) Amyloidogenic role of cytokine TGF-$\beta$1 in transgenic mice and in Alzheimer's disease. *Nature* **389:** 603–606.
45. Martel CL, Mackic JB, Matsubara E, et al. (1997) Isoform-specific effects of apolipoproteins E2, E3, and E4 on cerebral capillary sequestration and blood-brain barrier transport of circulating Alzheimer's amyloid $\beta$. *J. Neurochem.* **69:** 1995–2004.
46. Zlokovic BV, Martel CL, Mackic JB, et al. (1994) Brain uptake of circulating apolipoproteins J and E complexed to Alzheimer's amyloid $\beta$. *Biochem. Biophys. Res. Commun.* **205:** 1431–1437.
47. Mahley RW, Ji ZS. (1999) Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* **40:** 1–16.
48. Snow AD, Sekiguchi R, Nochlin D, et al. (1994) An important role of heparan sulfate proteoglycan (Perlecan) in a model system for the deposition and persistence of fibrillar A$\beta$-amyloid in rat brain. *Neuron* **12:** 219–234.
49. Naslund J, Thyberg J, Tjernberg LO, et al. (1995) Characterization of stable complexes involving apolipoprotein E and the amyloid $\beta$ peptide in Alzheimer's disease brain. *Neuron* **15:** 219–228.
50. Paka L, Kako Y, Obunike JC, Pillarisetti S.

(1999) Apolipoprotein E containing high density lipoprotein stimulates endothelial production of heparan sulfate rich in biologically active heparin-like domains. A potential mechanism for the anti-atherogenic actions of vascular apolipoprotein e. *J. Biol. Chem.* **274:** 4816–4823.

51. Castillo GM, Lukito W, Wight TN, Snow AD. (1999) The sulfate moieties of glycosaminoglycans are critical for the enhancement of $\beta$-amyloid protein fibril formation. *J. Neurochem.* **72:** 1681–1687.
52. Chapman PF, White GL, Jones MW, et al. (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat. Neurosci.* **2:** 271–276.
53. Mayeux R, Tang MX, Jacobs DM, et al. (1999) Plasma amyloid $\beta$-peptide 1–42 and incipient Alzheimer's disease. *Ann. Neurol.* **46:** 412–416.
54. Jack CRJ, Petersen RC, Xu YC, et al. (1999) Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology* **52:** 1397–1403.
55. Sparks DL. (1997) Coronary artery disease, hypertension, ApoE, and cholesterol: a link to Alzheimer's disease? *Ann. N. Y. Acad. Sci.* **826:** 128–146.
56. Swan GE, DeCarli C, Miller BL, et al. (1998) Association of midlife blood pressure to late-life cognitive decline and brain morphology. *Neurology* **51:** 986–993.