

Human Apolipoprotein E4 Alters the Amyloid- β 40:42 Ratio and Promotes the Formation of Cerebral Amyloid Angiopathy in an Amyloid Precursor Protein Transgenic Model

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Alzheimer's disease (AD) is characterized by the aggregation and deposition of the normally soluble amyloid- β ($A\beta$) peptide in the extracellular spaces of the brain as parenchymal plaques and in the walls of cerebral vessels as cerebral amyloid angiopathy (CAA). CAA is a common cause of brain hemorrhage and is found in most patients with AD. As in AD, the $\epsilon 4$ allele of the apolipoprotein E (apoE) gene (*APOE*) is a risk factor for CAA. To determine the effect of human apoE on CAA *in vivo*, we bred human *APOE3* and *APOE4* "knock-in" mice to a transgenic mouse model (Tg2576) that develops amyloid plaques as well as CAA. The expression of both human apoE isoforms resulted in a delay in $A\beta$ deposition of several months relative to murine apoE. Tg2576 mice expressing the more fibrillogenic murine apoE develop parenchymal amyloid plaques and CAA by 9 months of age. At 15 months of age, the expression of human apoE4 led to substantial CAA with very few parenchymal plaques, whereas the expression of human apoE3 resulted in almost no CAA or parenchymal plaques. Additionally, young apoE4-expressing mice had an elevated ratio of $A\beta$ 40:42 in brain extracellular pools and a lower 40:42 ratio in CSF, suggesting that apoE4 results in altered clearance and transport of $A\beta$ species within different brain compartments. These findings demonstrate that, once $A\beta$ fibrillogenesis occurs, apoE4 favors the formation of CAA over parenchymal plaques and suggest that molecules or treatments that increase the ratio of $A\beta$ 40:42 may favor the formation of CAA versus parenchymal plaques.

Key words: Alzheimer's disease; apolipoprotein E; cerebral amyloid angiopathy; amyloid- β ; ratio; transgenic models

Introduction

Alzheimer's disease (AD) is characterized by the extracellular aggregation and deposition of the normally soluble amyloid- β ($A\beta$) peptide in the form of diffuse and fibrillar plaques. $A\beta$ can also deposit in the walls of cerebral vessels, which is known as cerebral amyloid angiopathy (CAA). The $\epsilon 4$ allele of the apolipoprotein E (apoE) gene (*APOE*) is a risk factor for developing both AD and CAA (Schmechel et al., 1993; Greenberg et al., 1995; Nicoll et al., 1997). Conversely, although the $\epsilon 2$ allele is protective for AD, it is associated with an increased risk factor for developing hemorrhage when CAA is present (Nicoll et al., 1996, 1997; Greenberg et al., 1998). The exact molecular mechanism by which apoE affects AD or CAA is not completely clear, although increasing evidence

suggests that this is likely to be related in large part to the ability of apoE to act as an $A\beta$ chaperone (Wisniewski et al., 1997). apoE is a 299 aa protein secreted at high levels in the liver and the brain (Mahley, 1988). In the brain, it is secreted in unique high-density lipoprotein-like particles predominantly by glial cells (Pitas et al., 1987). apoE has been shown to bind directly to the $A\beta$ peptide and influence its fibrillogenesis and clearance *in vitro* (Strittmatter et al., 1993; Wisniewski et al., 1993; LaDu et al., 1994; Munson et al., 2000) and *in vivo* (Naslund et al., 1995; Wisniewski et al., 1995; Ghersi-Egea et al., 1996; Permann et al., 1997; Russo et al., 1998; Shibata et al., 2000; Ji et al., 2001; DeMattos et al., 2004). apoE has also been shown to be critically important for the formation of fibrillar $A\beta$ (Congo red or thioflavine positive) in brain parenchyma and CAA *in vivo* (Bales et al., 1997; Holtzman et al., 2000b; Fryer et al., 2003).

The $A\beta$ peptide is 38–43 aa in length and is generated from proteolytic processing of the amyloid- β precursor protein (APP), and the most common species is $A\beta_{40}$ and $A\beta_{42}$. The Tg2576 [Swedish mutation in the amyloid precursor protein (APP^{sw})] transgenic mouse model of AD develops parenchymal plaque pathology and prominent CAA beginning at 9 months of age

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(Hsiao et al., 1996). In the absence of endogenous murine apoE, APPsw mice still develop A β deposits, but the vast majority of the plaques are nonfibrillar, and the formation of CAA and CAA-associated hemorrhage is completely absent even at very old ages (Holtzman et al., 2000b; Fryer et al., 2003). Although murine apoE is homologous to human apoE, it differs by ~25% at the amino acid level. To further our understanding of the effects of human apoE isoforms on A β metabolism and CAA *in vivo*, we used APPsw mice expressing human apoE3 or apoE4 under the control of endogenous apoE regulatory elements (“knock-in” mice). These studies demonstrate early effects of apoE4 on increasing the A β 40:42 ratio and demonstrate that, once A β fibrillogenesis occurs, apoE4 results in a shift of A β deposition from brain parenchyma to the vasculature.

Materials and Methods

Animals and tissue preparation. The production, genotyping, and background strain (C57BL/6 \times SJL) of APPsw murine *ApoE*^{+/+} and APPsw murine *ApoE*^{-/-} mice used in this study have been described previously (Hsiao et al., 1996; Holtzman et al., 2000b). To determine the role of human apoE3 and apoE4 on CAA, we used mice in which the endogenous murine *ApoE* gene is replaced with the human *APOE3* or *APOE4* gene (knock-in mice). The production and genotyping of human *APOE3* and *APOE4* knock-in mice has been described previously (Sullivan et al., 1997). We bred *APOE3* and *APOE4* knock-in mice onto a C57BL/6 \times SJL background so that all of the mice used in this study were on an identical genetic background. APPsw mice overexpress a human APP₆₉₅ transgene containing the familial Swedish AD mutations at positions 670/671 under the control of the prion promoter and were a generous gift from Dr. K. Hsiao-Ashe (University of Minnesota, Minneapolis, MN). Animals were anesthetized with pentobarbital (150 mg/kg, i.p.) and perfused transcardially with 0.1 M PBS containing heparin (3 U/ml), pH 7.4, for 10 min. All of the mice used in this study were well perfused, as determined in the remaining hemibrain during histological assessment. For 15-month-old animals, one hemibrain was immersion-fixed overnight at 4°C in PBS containing 4% paraformaldehyde. After fixation, the brain was cryoprotected in PBS containing 30% sucrose at 4°C. The cerebellum of the remaining hemibrain was removed, and cerebral vessels were isolated from the forebrain, as described previously (Fryer et al., 2003). All experimental protocols used were approved by the Animal Studies Committee at Washington University.

Histological and biochemical analysis. Coronal sections (50 μ m) were cut on a freezing-sliding microtome, mounted on Superfrost Plus slides (Fisher Scientific, Houston, TX), and permeabilized for 30 min at room temperature with PBS containing 0.25% Triton X-100. To visualize fibrillar A β plaques and CAA, sections were stained with thioflavine S or the Congo red derivative X-34 dye in 40% ethanol in PBS (a generous gift from Dr. W. Klunk, University of Pittsburgh, Pittsburgh, PA). Sections were quickly rinsed twice in 40% ethanol in PBS and twice in PBS before being mounted with Vectashield (Vector Laboratories, Burlingame, CA). X-34 has the advantage of a greater signal-to-noise ratio, as well as a narrow emission spectra, compared with thioflavine S (Styren et al., 2000). For PBS-soluble extracts, tissue was briefly Dounce homogenized in ice-cold PBS containing protease inhibitors and immediately spun at 20,000 \times g for 5 min. A β _{1–40} and A β _{1–42} were quantified by ELISA with specific monoclonal antibodies, as described previously (Cirrito et al., 2003). Total tissue cholesterol was determined using the Amplex Red cholesterol kit (Sigma, St. Louis, MO).

apoE ELISA. Plates (96 well) were coated overnight with the monoclonal antibody WU-E4 (Krul et al., 1988) at a concentration of 10 μ g/ml in 35 mM NaHCO₃, 16 mM NaCO₃, 0.02% NaN₃, pH 9.6. Plates were washed and blocked for 1 h at 37°C with 1% dry milk–PBS. Plates were washed again, and samples and standards were loaded in ELISA buffer (0.5% BSA–PBS and 0.025% Tween 20) and incubated overnight at 4°C. Human apoE isolated from plasma β -very-low-density lipoprotein was used as standard (BIODESIGN International, Saco, ME). Plates were washed again and incubated for 90 min at 37°C with goat anti-apoE

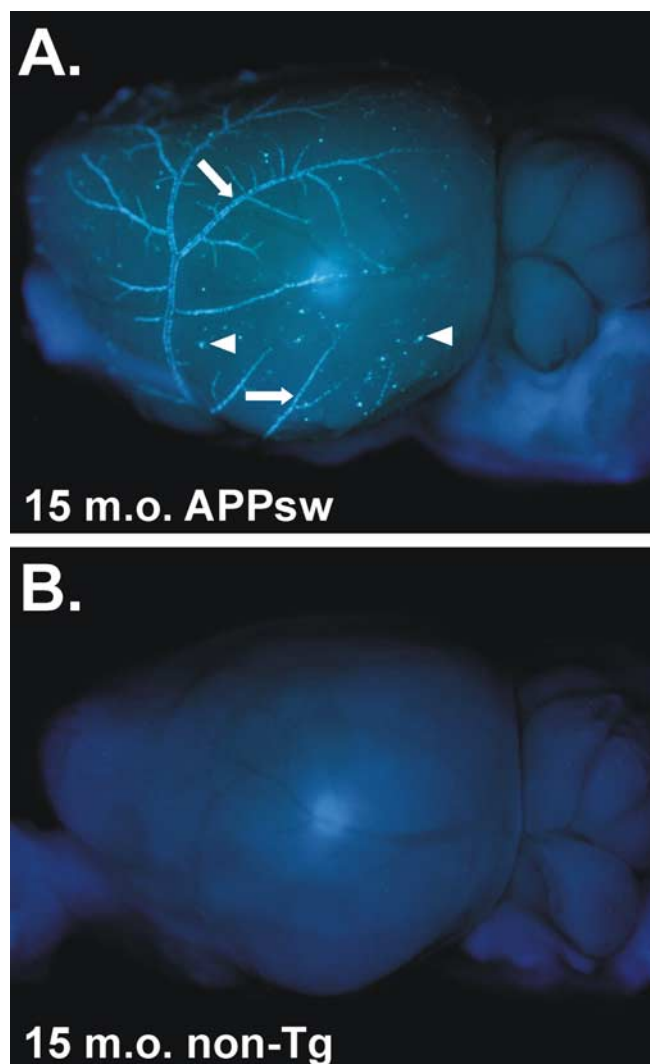


Figure 1. *Ex vivo* image of an APPsw brain demonstrating extensive CAA. **A**, A 15-month-old (15 m.o.) APPsw mouse brain stained with X-34 and imaged with UV epifluorescence to denote fibrillar amyloid. Extensive CAA in leptomenigeal vessels can be seen on the cortical surface (arrows) as well as on plaques near the cortical surface (arrowheads). **B**, A 15-month-old non-transgenic (non-Tg) mouse brain stained with X-34, demonstrating the specificity of the stain.

antisera at a 1:500 dilution (Calbiochem, La Jolla, CA) in ELISA buffer. Plates were washed again and incubated with HRP-conjugated horse anti-goat antibody (Vector Laboratories) at a 1:5000 dilution in ELISA buffer. Plates were washed and developed with tetramethylbenzidine substrate (Sigma) and monitored for absorbance at 650 nm. The Student's *t* test was used to compare apoE levels in human apoE3- and apoE4-expressing mice.

Quantitation of CAA and A β load. The percentage of cross-sectional area covered by CAA vessels (percentage of CAA load), as defined by X-34-positive vessels, was quantified using unbiased stereological principles, as described previously (Fryer et al., 2003). Surface and penetrating CAA load, as well as amyloid plaque load, was determined in the cortex and overlying leptomenigeal vessels immediately dorsal to the striatum in four sections, each separated by 300 μ m. Stereo Investigator image-analysis software (MicroBrightField, Williston, VT) was used to quantify percentage of CAA load, as well as amyloid plaque load, using Cavalieri's point-counting method. One-way ANOVA, followed by a *post hoc* Tukey's *t* test, was used to compare APPsw mice expressing murine apoE, human apoE3, or human apoE4. Because the data were not normally distributed, the Mann–Whitney *t* test was used to compare deposits in human apoE3- or apoE4-expressing mice.

Isolation of cerebral vessels, parenchymal tissue, and CSF. The isolation

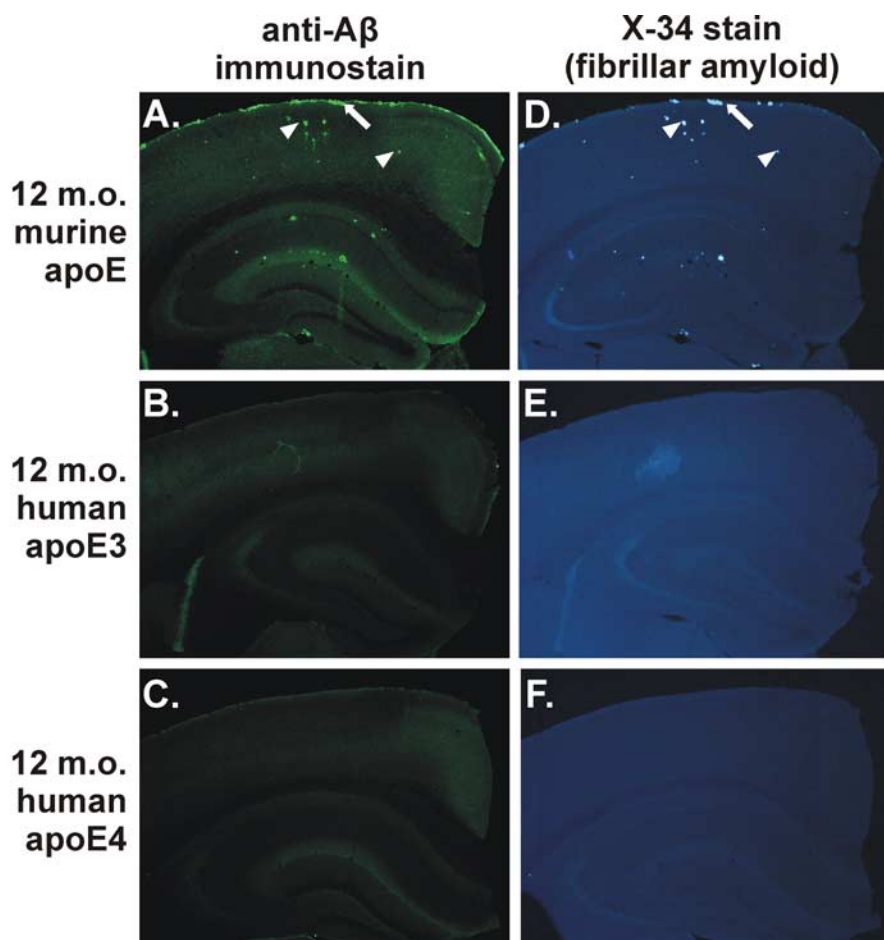


Figure 2. Expression of human apoE3 or apoE4 delays deposition compared with murine apoE in the APPsw model at 12 months of age. *A, D*, Anti- $A\beta$ immunostaining with monoclonal antibody m3D6 (*A*) or X-34 staining (*D*) to denote fibrillar amyloid of APPsw mice expressing endogenous murine apoE demonstrating CAA (arrows) and parenchymal plaques (arrowheads). However, 12-month-old (12 m.o.) APPsw mice expressing human apoE3 (*B, E*) or apoE4 (*C, F*) had little or no detectable plaques.

of cerebral vessels and parenchymal tissue was performed exactly as described previously (Fryer et al., 2003). Murine CSF was isolated as described previously (DeMattos et al., 2002).

Results

APPsw mice develop prominent amyloid plaques and CAA: human apoE delays $A\beta$ deposition

In this study, we used human *APOE* knock-in mice to study the effects of human apoE3 and apoE4 as well as murine apoE on $A\beta$ deposition in the APPsw mouse model of AD. In this model, $A\beta$ is deposited predominantly as fibrillar $A\beta$ plaques (i.e., thioflavine S- or X-34-positive amyloid). In addition to parenchymal amyloid plaques, this model also develops substantial CAA. An *ex vivo* amyloid stain demonstrates the extensive CAA in the leptomeningeal vessels on the surface of the brain (Fig. 1*A*) in this model, which is perhaps underappreciated when simply histologically examining individual brain tissue sections. In the APPsw model, $A\beta$ begins to deposit as parenchymal plaques and CAA at ~9 months of age and is present in all of the APPsw mice by 12 months of age (Fig. 2*A, D*) (Hsiao et al., 1996). We found previously that the expression of human apoE isoforms in another APP transgenic mouse model (PDAPP) with an APP^{V717F} mutation (Holtzman et al., 2000a; Fagan et al., 2002) resulted in a delay in deposition of several months. In this APPsw model, we also found that the expression of human apoE under the control of

endogenous regulatory elements results in a delay of several months or longer in $A\beta$ deposition compared with animals expressing endogenous murine apoE (Fig. 2*B, C, E, F*).

apoE4 shifts amyloid deposition from parenchyma to vessels

APPsw mice expressing human apoE4 did begin to deposit $A\beta$ between 12 and 15 months of age. Seven of 11 APPsw mice expressing human apoE4 had $A\beta$ deposition at 15 months of age, whereas 2 of 12 APPsw mice expressing human apoE3 had $A\beta$ deposition. Strikingly, the $A\beta$ deposited in the presence of apoE4 was almost exclusively in cerebral vessels and was all fibrillar, with very few parenchymal plaques, at 15 months of age (Fig. 3*C, F*). This is in contrast to the mixed pathology of parenchymal plaques and CAA in APPsw mice expressing endogenous murine apoE (Fig. 3*A, D*). APPsw mice expressing human apoE3 developed very few plaques at this age (Fig. 3*B, E*), with no histologically detectable deposition of $A\beta$ in the majority of these animals. To more thoroughly analyze the deposition of $A\beta$ in brain parenchyma versus CAA, we performed a stereological analysis to quantify area covered by CAA, as well as fibrillar parenchymal plaques. The expression of apoE4 resulted in a marked and significant increase in CAA, as well as parenchymal amyloid plaques, compared with mice expressing apoE3 (Fig. 4). Although murine apoE resulted in the greatest amount of amyloid deposition compared with either of the human apoE isoforms, the ratio of amyloid deposition in vessels versus parenchyma was much lower in the presence of murine apoE (3:1 ratio) versus apoE4 (21:1 ratio). Strikingly, virtually all $A\beta$ deposits in the apoE4-expressing mice at 15 months of age were in the form of CAA (Fig. 4). The expression of apoE4 resulting in mostly CAA at 15 months of age is not merely attributable to an overall delay in parenchymal $A\beta$ deposition, because 12-month-old APPsw mice expressing murine apoE develop comparable levels of parenchymal plaques and CAA (Fig. 2*A, D*). Interestingly, although APPsw mice expressing apoE4 had substantial levels of CAA, the expression of both apoE3 and apoE4 in APPsw mice resulted in a dramatic reduction in the amount of fibrillar parenchymal plaques in the cortex compared with APPsw mice expressing murine apoE (Fig. 4*D*).

To further examine the amount and type of $A\beta$ deposited in APPsw mice expressing human apoE3 or apoE4 at 15 months of age, we isolated cerebral vessels and brain parenchymal tissue to measure the absolute level of $A\beta$ by ELISA. As would be expected from the histological quantitation and from previous biochemical studies of CAA (Joachim et al., 1988; Suzuki et al., 1994; Fryer et al., 2003), cerebral vessels isolated from 15-month-old APPsw mice expressing human apoE4 had substantially more $A\beta_{1-40}$ and $A\beta_{1-42}$ than human apoE3-expressing mice (Fig. 5*A, B*). Also reflective of the histological quantitation, brain parenchymal lev-

els of A β in 15-month-old APPsw mice expressing human apoE4 were considerably higher than in human apoE3-expressing mice (Fig. 5C,D).

apoE4 alters the A β 40:42 ratio in brain tissue and CSF in young APPsw mice

Given the dramatic effect of human apoE isoforms and species on A β -related pathology at 15 months of age, we examined young mice at 3 months of age, an age well before the development of A β deposition, for changes that may explain the shift from mixed CAA/parenchymal pathology to predominantly CAA by apoE4 expression. We examined the levels of apoE and A β in both cortical PBS extracts (as a reflection of the extracellular parenchymal pool) as well as in CSF. We found that APPsw mice expressing human apoE4 had significantly higher levels of A β_{1-40} in PBS extracts compared with mice expressing murine apoE (Fig. 6A). A β_{1-42} levels were also slightly lower in these PBS extracts of APPsw mice expressing human apoE4, but this did not reach statistical significance (Fig. 6A). A β_{1-40} levels in CSF were slightly lower in APPsw mice expressing human apoE4, but this also did not reach statistical significance (Fig. 6B). However, there was a significantly higher ratio of A β 40:42 in PBS extracts of cortical tissue of mice expressing apoE4 compared with mice expressing endogenous murine apoE (Fig. 6C). Interestingly, this ratio was reversed in CSF, and human apoE4-expressing mice had a significantly lower A β 40:42 ratio than the other genotypes, suggesting that apoE4 results in a less-efficient transport of A β between brain parenchymal tissues to other CNS compartments such as CSF (Fig. 6D). These results also highlight the importance of examining the A β 40:42 ratio, in addition to absolute levels of different A β peptides, and suggest that a higher 40:42 ratio in the brain predisposes it to CAA. The level of A β_{40} , A β_{42} , or the ratio of A β 40:42 did not differ between any of the genotypes in the plasma of these mice (data not shown). This highlights the observation that different apoE-dependent mechanisms seem to be involved in A β transport within the CNS versus peripheral circulation. The levels of apoE in apoE3- and apoE4-expressing mice were also not significantly different in either cortex (Fig. 7A) or CSF (Fig. 7B), indicating that differences in the level of apoE do not account for the pathological differences observed between APOE3 and APOE4 knock-in mice. It was not possible to directly compare the levels of murine to human apoE in an ELISA format, because the murine and human apoE standards are not identical, and the detection antibody does not recognize murine and human apoE equally. Additionally, we found no differences in brain cholesterol in mice expressing murine apoE or human apoE3 or apoE4, suggesting that apoE isoform and species did not influence brain cholesterol to somehow influence A β (Fig. 7C).

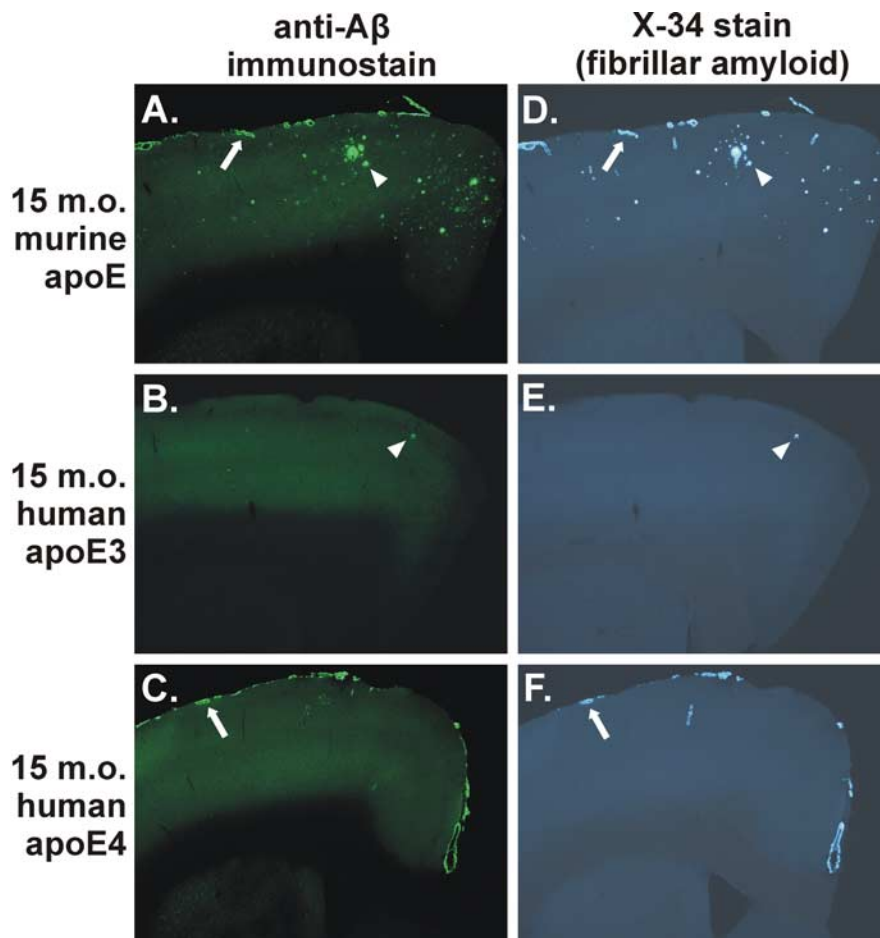


Figure 3. Expression of apoE determines the level of CAA and parenchymal plaque pathology in an isoform-dependent manner in the 15-month-old (15 m.o.) APPsw model. **A–F**, Anti-A β immunostaining with m3D6 antibody (**A–C**) or X-34 staining (**D–F**) to visualize fibrillar A β deposits demonstrates CAA (arrows) and parenchymal plaques (arrowheads). **A, D**, APPsw mice expressing endogenous murine apoE had both CAA and parenchymal plaques. **B, C, E, F**, APPsw mice expressing human apoE3 (**B, E**) had only occasional A β deposits, whereas APPsw mice expressing human apoE4 (**C, F**) had extensive CAA with infrequent parenchymal plaques.

Discussion

Although much is known about the factors that lead to the deposition of A β in brain parenchyma, much less is known about the mechanisms of A β deposition in cerebral vessels. We used the APPsw mouse model of AD and human APOE knock-in mice to explore the effects of human apoE expression on A β deposition in brain parenchyma and cerebral vessels. As we saw previously in another mouse model of AD (Holtzman et al., 1999, 2000a), expression of human apoE isoforms resulted in a marked delay in deposition of A β until ~15 months of age. Strikingly, the expression of human apoE4 in APPsw mice at 15 months of age led to a shift in A β deposits (relative to the expression of endogenous murine apoE), resulting in substantial CAA with very little parenchymal plaque deposition. Expression of human apoE3 resulted in almost no A β deposition at this age. Reflective of these histological observations, biochemical analysis of A β levels in cerebral vessels of aged mice showed a dramatic increase in the level of A β_{1-40} and A β_{1-42} in APPsw mice expressing human apoE4 compared with human apoE3-expressing mice. Biochemical analysis of young mice well before A β deposition showed no differences in the level of human apoE in the brain or CSF. There were also no differences in brain cholesterol in mice expressing

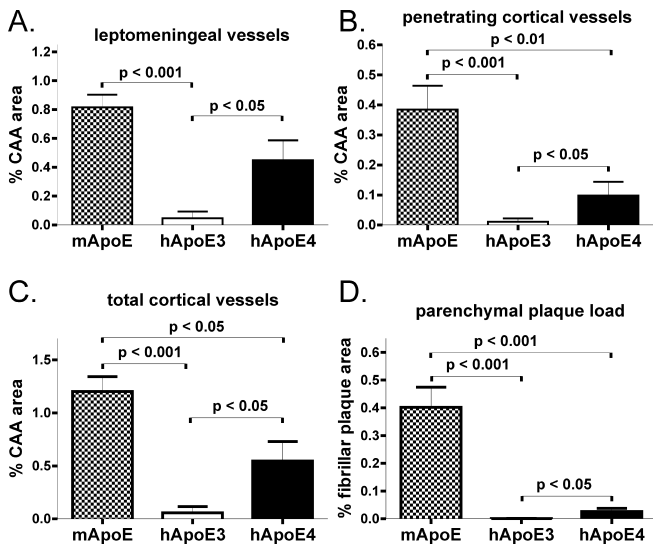


Figure 4. Stereological quantitation of CAA and fibrillar plaque load. **A–C**, In 15-month-old APPsw mice, expression of human apoE4 (hApoE) and murine apoE (mApoE) resulted in significantly greater CAA load in leptomeningeal vessels (**A**), penetrating cortical vessels (**B**), and total cortical vessels (**C**) and parenchymal plaque load compared with mice expressing human apoE3. **B–D**, Expression of murine apoE also resulted in significantly higher levels of penetrating (**B**), total CAA (**C**), and parenchymal plaque load (**D**) compared with expression of human apoE4. **D**, Expression of both human apoE3 and apoE4 resulted in a dramatic reduction in parenchymal amyloid plaque load compared with mice expressing murine apoE. Error bars represent SEM.

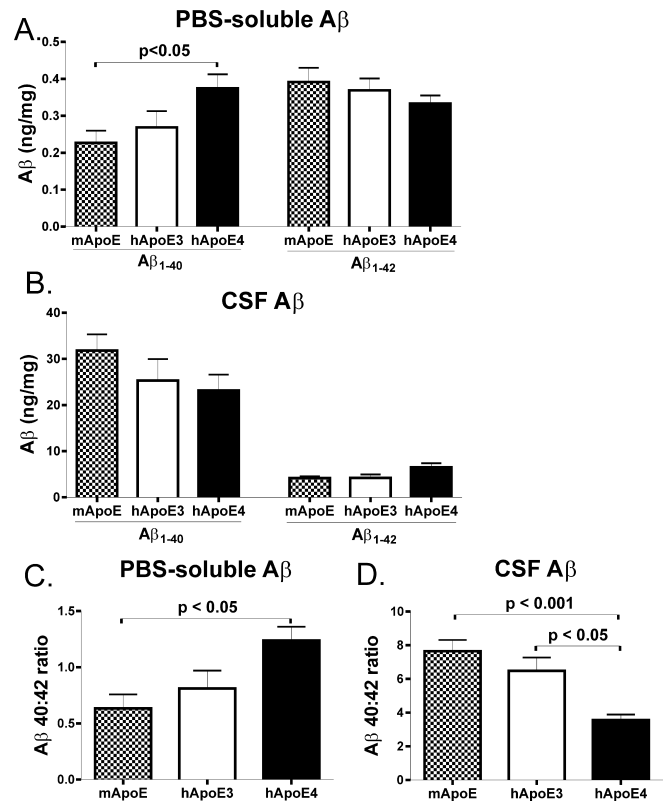


Figure 6. Expression of human apoE4 (hApoE4) alters the ratio of A β 40:42 in extracellular pools of the CNS. **A**, The level of A β_{1-40} in PBS-soluble brain extracts was significantly higher in APPsw mice expressing human apoE4 compared with mice expressing murine apoE (mApoE). A β_{1-42} levels were slightly, but not significantly, lower in APPsw mice expressing human apoE4 compared with mice expressing murine apoE. **B**, The level of A β_{1-40} in the CSF was slightly, but not significantly, lower in APPsw mice expressing human apoE4 compared with mice expressing murine apoE. Also, A β_{1-42} levels were slightly, but not significantly, higher in APPsw mice expressing human apoE4 compared with mice expressing murine apoE. **C**, The A β 40:42 ratio in PBS-soluble extracts was significantly higher in APPsw mice expressing human apoE4 compared with murine apoE. **D**, However, the A β 40:42 ratio in the CSF was significantly lower in APPsw mice expressing human apoE4 compared with mice expressing human apoE3 or murine apoE. Error bars represent SEM.

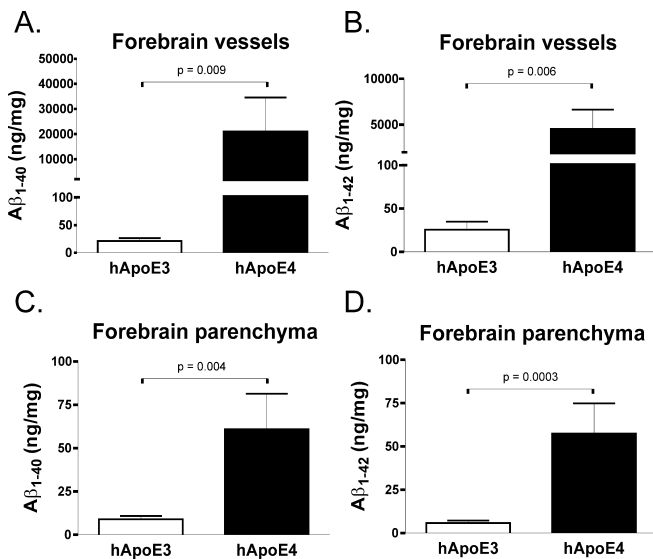


Figure 5. Fifteen-month-old APPsw mice expressing human apoE4 (hApoE4) had dramatically more deposition of A β in cerebral vessels than mice expressing human apoE3. **A, B**, Human apoE3-expressing mice deposited 21.26 ± 5.097 ng/mg A β_{1-40} and 25.58 ± 9.282 ng/mg A β_{1-42} in cerebral vessels, whereas human apoE4-expressing mice deposited $21,040 \pm 13,530$ ng/mg A β_{1-40} and 4535 ± 2094 ng/mg A β_{1-42} in cerebral vessels. **C, D**, Additionally, human apoE3-expressing mice deposited 8.493 ± 1.918 ng/mg A β_{1-40} and 5.827 ± 1.421 ng/mg A β_{1-42} in brain parenchymal tissue, whereas human apoE4-expressing mice deposited 60.89 ± 20.53 ng/mg A β_{1-40} and 57.38 ± 17.48 ng/mg A β_{1-42} in brain parenchymal tissue. Error bars represent SEM.

human apoE3, human apoE4, or endogenous murine apoE. Interestingly, APPsw mice expressing human apoE4 had a significant increase in the A β 40:42 ratio in extracellular brain pools, whereas this ratio was reversed in CSF. A β levels or 40:42 ratios were not different between any of the genotypes in the plasma of

these young mice, further indicating that apoE-dependent A β clearance seems to occur by different mechanisms within the CNS versus peripheral circulation. Thus, there are two main effects of human apoE expression on A β deposition in APPsw mice: (1) a delay in A β deposition by both human apoE3 and apoE4 (likely attributable to the fact that murine apoE is more efficient in facilitating aggregation of A β into fibrillar forms) and (2) a striking redistribution of A β deposition to the cerebral vasculature once A β fibrillogenesis begins in the presence of apoE4 (likely facilitated by an increased A β 40:42 ratio present at young ages). Together, these data suggest that apoE isoforms differentially alter the clearance and transport of A β species within brain extracellular space and that, once A β fibrillogenesis occurs, an increased 40:42 ratio results in a shift of A β deposition from the brain parenchyma to vessels.

In humans, APOE4 is associated with larger amounts of CAA in patients with AD, and some APOE4 homozygous individuals examined at older ages have been shown to have substantial CAA (Schmechel et al., 1993; Vidal et al., 2000). In a study of 125 AD cases, the severity of CAA was strongly associated with the number of APOE4 alleles, whereas parenchymal A β was independent of APOE genotype (Chalmers et al., 2003). Interestingly, cases

with severe CAA had a significantly lower parenchymal $A\beta$ load (Chalmers et al., 2003). Although *APOE2* seems to protect against developing AD, it is a known risk factor for CNS hemorrhage if CAA is present (Nicoll et al., 1996, 1997; Greenberg et al., 1998). Additional studies in AD mouse models expressing human apoE2 will be useful in determining the mechanism of this CAA-associated hemorrhage, and we are in the process of generating these mice. Despite these genetic associations discovered over a decade ago, little is known about the molecular mechanisms by which apoE influences the development of CAA. Previously, we demonstrated a critical role of murine apoE in CAA. APPsw mice lacking endogenous murine apoE do not develop CAA even up to 24 months of age, despite significant parenchymal plaque deposition, some of which is fibrillar (Fryer et al., 2003). Together with the results presented herein, these data suggest that apoE may play as prominent a role in the deposition of $A\beta$ in cerebral vessels as it does in brain parenchyma.

Cerebral vessels in both human cases of CAA as well as mouse models with extensive CAA have been shown to have high levels of $A\beta_{1-40}$ deposited compared with $A\beta_{1-42}$ (Joachim et al., 1988; Prelli et al., 1988; Suzuki et al., 1994; Alonzo et al., 1998; McCarron et al., 2000; Fryer et al., 2003; Ingelsson et al., 2004). Additionally, as with the APPsw mice used in this study, familial AD patients with the Swedish APP 670/671 mutation have substantial CAA (Lannfelt et al., 1994). Recent evidence has also suggested that the $A\beta$ 40:42 ratio may be a key pathologic factor favoring deposition of $A\beta$ in cerebral vessels versus brain parenchyma. APP transgenic mice expressing the APP mutation that results in hereditary cerebral hemorrhage with amyloidosis [i.e., E22Q (Dutch type)] have a high $A\beta$ 40:42 ratio and develop extensive CAA with few parenchymal plaques (Herzig et al., 2004). However, when this model was bred to a presenilin transgenic mouse model (PS45) harboring a mutation favoring generation of $A\beta_{1-42}$ over $A\beta_{1-40}$, young mice had a low $A\beta$ 40:42 ratio, and the pathology in aged mice was shifted from CAA to parenchymal plaque deposition (Herzig et al., 2004). Because the expression of apoE4 versus apoE3 and murine apoE in APPsw mice results in a high 40:42 ratio in brain parenchymal tissue and the later development of a significantly elevated ratio of CAA to parenchymal plaques, this supports the hypothesis that a high $A\beta$ 40:42 ratio favors the formation of CAA over parenchymal plaque pathology. The interesting aspect of this finding in relation to apoE4 is that a preponderance of current evidence suggests that the effect of apoE on $A\beta$ metabolism, including the 40:42 ratio, is via apoE acting as an $A\beta$ chaperone and not by affecting $A\beta$ production from APP.

We showed previously that, although apoE clearly affects the clearance of $A\beta$ *in vivo*, additional studies will be necessary to determine the molecular mechanisms underlying this effect. Several *in vitro* studies suggest that apoE influences cellular $A\beta$ clearance, and our recent *in vivo* study suggests that apoE in some way directly influences the half-life of soluble $A\beta$ in the interstitial fluid of the brain (DeMattos et al., 2004). The finding that apoE4-expressing mice have a significantly higher $A\beta$ 40:42 ratio in the PBS-soluble brain fraction and a significantly lower 40:42 ratio in the CSF, compared with mice expressing apoE3 and murine apoE, also strongly supports the idea that apoE can directly influence soluble $A\beta$ metabolism by altering its clearance and trans-

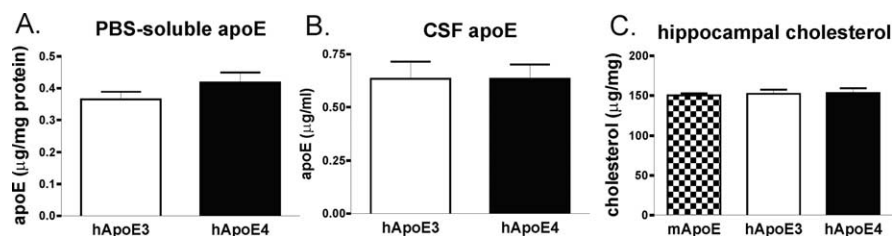


Figure 7. Expression of murine apoE (mApoE) or human apoE3 (hApoE3) or apoE4 (hApoE4) does not alter apoE levels or total brain cholesterol. **A, B**, Levels of human apoE were not significantly different in PBS-soluble brain extracts of cortical tissue (**A**) or in CSF (**B**). **C**, Additionally, the level of hippocampal cholesterol did not significantly differ among mice expressing murine apoE, human apoE3, or human apoE4. Error bars represent SEM.

port between brain compartments. Additionally, the fact that APP transgenic mice that are *ApoE*^{-/-} develop $A\beta$ deposits in a different anatomical distribution (Bales et al., 1997; Holtzman et al., 1999; Irizarry et al., 2000) also suggests that apoE is involved in extracellular $A\beta$ transport. The clearance of $A\beta$ from brain parenchyma has been proposed to occur along periarterial interstitial fluid drainage pathways (Weller et al., 1998). Interestingly, in recent *in vivo* imaging experiments with APPsw mice, we find that all CAA is present only in arterioles and not venules (D. M. Holtzman, unpublished observations). The local environment along these pathways has abundant molecules known to bind apoE, such as heparan sulfate proteoglycans and low-density lipoprotein receptor family members (Shibata et al., 2000; Strittmatter and Bova Hill, 2002). It is possible that apoE- $A\beta$ complexes are transported from brain parenchymal tissue to these interstitial fluid drainage pathways in which they bind to apoE-binding molecules, and the local environment promotes the fibrillogenesis of $A\beta$. Other secreted molecules present in the brain extracellular space, such as TGF- β , also likely contribute to the pathogenesis of CAA (Wyss-Coray et al., 1997). For example, overexpression of TGF- β increases CAA in APP transgenic mice, and this may be attributable to the upregulation of extracellular matrix proteins (Wyss-Coray et al., 1997, 2000). Finally, the results presented here suggest that caution should be taken in AD therapies that may subtly alter the $A\beta$ 40:42 ratio in brain tissue, either intentionally or unintentionally, because they may be effective in reducing parenchymal plaque deposition at the expense of developing CAA (Davis et al., 2004; Deane et al., 2004).

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