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Deletion of *Abca1* Increases Aβ Deposition in the PDAPP Transgenic Mouse Model of Alzheimer Disease*

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Apolipoprotein E (apoE) genotype has a major influence on the risk for Alzheimer disease (AD). Different apoE isoforms may alter AD pathogenesis via their interactions with the amyloid β-peptide (Aβ). Mice lacking the lipid transporter ABCA1 were found to have markedly decreased levels and lipidation of apoE in the central nervous system. We hypothesized that if *Abca1*$^{-/-}$ mice were bred to the PDAPP mouse model of AD, PDAPP *Abca1*$^{-/-}$ mice would have a phenotype similar to that of PDAPP *Apoe*$^{+/-}$ and PDAPP *Apoe*$^{-/-}$ mice, which develop less amyloid deposition than PDAPP *Apoe*$^{+/+}$ mice. In contrast to this prediction, 12-month-old PDAPP *Abca* $^{-/-}$ mice had significantly higher levels of hippocampal Aβ, and cerebral amyloid angiopathy was significantly more common compared with PDAPP *Abca1*$^{+/+}$ mice. Amyloid precursor protein (APP) C-terminal fragments were not different between *Abca1* genotypes prior to plaque deposition in 3-month-old PDAPP mice, suggesting that deletion of *Abca1* did not affect APP processing or Aβ production. As expected, 3-month-old PDAPP *Abca1*$^{-/-}$ mice had decreased apoE levels, but they also had a higher percentage of carbonate-insoluble apoE, suggesting that poorly lipidated apoE is less soluble *in vivo*. We also found that 12-month-old PDAPP *Abca1*$^{-/-}$ mice had a higher percentage of carbonate-insoluble apoE and that apoE deposits co-localize with amyloid plaques, demonstrating that poorly lipidated apoE co-deposits with insoluble Aβ. Together, these data suggest that despite substantially lower apoE levels, poorly lipidated apoE produced in the absence of ABCA1 is strongly amyloidogenic *in vivo*.

Apolipoprotein E (apoE)[2] genotype is a strong determinant of risk for Alzheimer disease (AD) and cerebral amyloid angiopathy (CAA) (1–3). Relative to subjects with the more common ε3 allele of apoE, subjects with one or more ε4 alleles have a higher risk for AD and CAA, whereas subjects with one or more ε2 alleles have a decreased risk for AD (2, 4). Evidence suggests that the mechanism by which different apoE alleles affect the pathogenesis of AD and CAA is by modifying interactions between apoE and the amyloid β-peptide (Aβ), which aggregates and deposits into the amyloid plaques that are thought to initiate AD and CAA pathogenesis (5, 6). ApoE may act as a chaperone for Aβ by binding the peptide and altering its conformation, thereby influencing its clearance and ability to aggregate (7). Evidence supporting the role of apoE as an Aβ chaperone comes from a wide range of experiments. ApoE isoforms and levels affect the aggregation, fibrillogenesis, clearance, and degradation of Aβ in cell-free, cell-based, and tissue-based experiments (6, 8–11).

Studies have also shown a profound effect of apoE on Aβ deposition and conformation *in vivo*. The PDAPP and Tg2576 transgenic mouse models of AD, which overexpress the human amyloid precursor protein (APP) containing AD-causing mutations, develop several aspects of AD-like pathology beginning at 6–9 months of age, including diffuse and fibrillar Aβ deposits, neuritic plaques, gliosis, and CAA (12, 13). When Tg2576 and PDAPP mice were bred to animals lacking murine apoE, the Tg2576 *Apoe*$^{-/-}$, and PDAPP *Apoe*$^{-/-}$ mice developed much less Aβ deposition, almost no fibrillar Aβ deposits or neuritic plaques, and no CAA (14–17). These effects were dose-dependent such that *Apoe*$^{+/-}$ mice had less than 50% as much Aβ-related pathology as *Apoe*$^{+/+}$ mice (14, 17). These findings demonstrate that murine apoE strongly promotes Aβ-related pathology *in vivo*. The influence of apoE type and the levels on Aβ behavior in many different systems supports the hypothesis that apoE affects the risk for AD via chaperone-like interactions with Aβ.

ABCA1 (ATP-binding cassette A1), a member of the ATP-binding cassette family of transporters, transfers cellular cholesterol and phospholipids onto lipid-poor apolipoproteins to form pre-HDL (18, 19). ABCA1-mediated transport of cellular cholesterol onto apolipoproteins is the rate-limiting step in the anti-atherosclerotic reverse cholesterol transport pathway, which allows the removal of excess cholesterol from tissues by HDL followed by delivery of cholesterol to the liver for excretion into bile acids (20). In humans, loss of function mutations in ABCA1 cause Tangier disease (21–24), which is characterized by accumulation of cholesterol in lymphatic tissues and increased catabolism of abnormally lipidated HDL, resulting in very low levels of plasma HDL and the HDL-associated apolipoproteins apoAI and apoAII (25, 26). *Abca1*$^{-/-}$ mice have a similar phenotype as patients with Tangier disease, with greatly decreased HDL and apoAI and accumulation of lipid in the lungs and other tissues (27).

Recently, both Wahrle *et al.* (28) and Hirsch-Reinshagen *et al.* (29) showed that *Abca1*$^{-/-}$ mice have very low levels of apoE in the central nervous system, which were ~20% of wild type in the cortex and 2% of wild type in the cerebrospinal fluid (CSF) (28). *In vitro* studies revealed that the primary cultures of astrocytes, the major producers of apoE in the central nervous system, secrete apoE in small, very poorly lipidated lipoprotein particles if they are derived from *Abca1*$^{-/-}$ mice (28). Particles derived from *Abca1*$^{-/-}$ mice contained only 0.69 μg of total cho-

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[2] The abbreviations used are: apoE, apolipoprotein E; Aβ, amyloid β-peptide; AD, Alzheimer disease; APP, amyloid precursor protein; CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; CTF, C-terminal fragment; ELISA, enzyme-linked immunosorbent assay; HDL, high density lipoprotein; LDLR, low density lipoprotein receptor; LXR, liver X receptor; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; MES, 4-morpholineethanesulfonic acid; LRP, low density lipoprotein receptors.

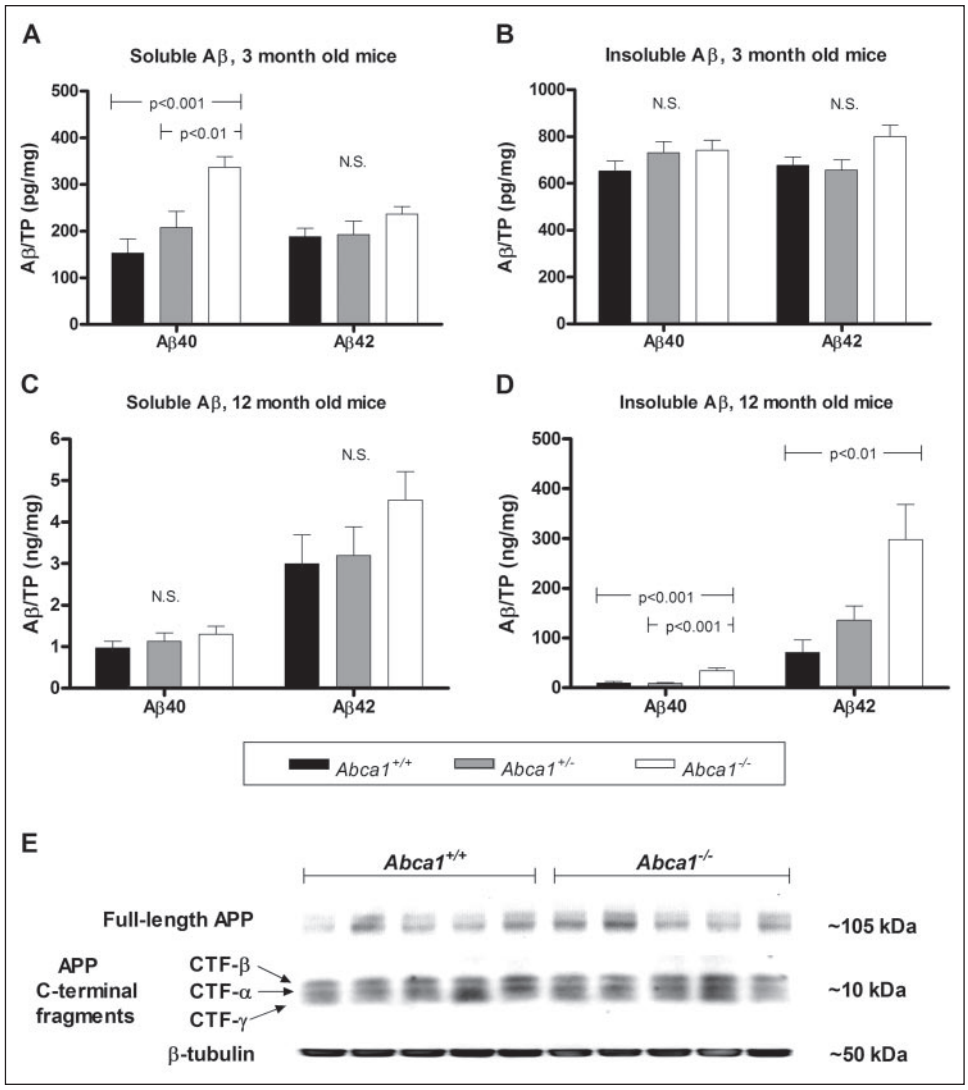

FIGURE 1. **Levels of Aβ, APP, and APP C-terminal fragments in PDAPP *Abca1* mice.** *A–D*, Aβ levels in hippocampi were measured by ELISA and normalized to total protein levels. Soluble Aβ was extracted using carbonate buffer. Insoluble Aβ was not extractable with carbonate buffer and required 5 M guanidine for extraction. In the 3-month-old group, $n = 8$ for PDAPP *Abca1*$^{+/+}$, $n = 11$ for PDAPP *Abca1*$^{+/-}$, and $n = 13$ for PDAPP *Abca1*$^{-/-}$ mice. In the 12-month-old group, $n = 12$ for PDAPP *Abca1*$^{+/+}$, $n = 9$ for PDAPP *Abca1*$^{+/-}$, and $n = 11$ for PDAPP *Abca1*$^{-/-}$ mice. *E*, the levels of APP and APP C-terminal fragments in cortex in $n = 5$ PDAPP *Abca1*$^{+/+}$ and $n = 5$ PDAPP *Abca1*$^{-/-}$ mice were measured by Western blot. β-Tubulin was used as a loading control.

lesterol/μg of apoE, whereas particles from *Abca1*$^{+/+}$ mice contained 2.3 μg of total cholesterol/μg of apoE. The decreased central nervous system apoE levels seen in the *Abca1*$^{-/-}$ mice likely result from rapid catabolism of the poorly lipidated apoE-containing HDL particles. The dramatic alterations in central nervous system of apoE produced by *Abca1* deletion provide an opportunity to determine how changes in apoE levels and lipidation influence Aβ metabolism *in vivo*.

In the present study, we bred *Abca1*$^{-/-}$ mice to a well characterized APP transgenic mouse model of AD (PDAPP) that develops age- and region-dependent AD-like pathology (15). Because *Abca1*$^{-/-}$ mice have a greatly decreased apoE, we hypothesized that PDAPP *Abca1*$^{-/-}$ mice would develop similar levels of pathology as aged PDAPP mice with either no apoE or 50% less apoE (PDAPP *Apoe*$^{-/-}$ or *Apoe*$^{+/-}$ mice), both of which have significantly lower levels of nonfibrillar and fibrillar Aβ deposits as well as less CAA. Most interestingly and contrary to our hypothesis, 12-month-old PDAPP *Abca1*$^{-/-}$ mice had increased parenchymal Aβ levels, amyloid deposition, and CAA. These results suggest that the poorly lipidated apoE formed in the absence of ABCA1 facilitates amyloidogenesis, even when present at low levels.

EXPERIMENTAL PROCEDURES

Animals and Tissue Collection—Mice heterozygous for an *Abca1* deletion gene on a DBA background were obtained from The Jackson Laboratory, Bar Harbor, ME (strain name, DBA/1-Abca1^{tm1Jdm}). Transgenic mice overexpressing human APP containing the V717F familial Alzheimer disease mutation on a C57Bl/6 background, referred to as PDAPP mice (12), were obtained from Lilly. The *Abca1*$^{+/-}$ and PDAPP mice were bred to one another for three generations to produce mice of all *Abca1* genotypes that were hemizygous for the PDAPP transgene. All mice were genotyped by PCR. Animals used for experiments were either 3 or 12 months old and were of the same generation. At the appropriate ages, the mice were anesthetized with pentobarbital, and CSF was collected from the cisterna magna as described (30), and the animals were perfused with phosphate-buffered saline/heparin (3 units/ml). The hippocampi and cortices were dissected from the brains and frozen on dry ice.

Aβ and ApoE ELISAs—Hippocampi were subjected to a serial extraction method using carbonate and guanidine buffers as described previously (31). Briefly, hippocampi were homogenized in 10 μl/mg carbonate buffer (100 mM sodium carbonate, 50 mM NaCl, protease inhibitors, pH 11.5) and centrifuged at 20,000 × *g* for 25 min. The carbonate-soluble supernatant was collected, and the pellet was re-homogenized with 700 μl of guanidine buffer (5 M guanidine, 50 mM Tris, protease inhibitors, pH 8.0). The homogenate was centrifuged at 20,000 × *g* for 25 min, and the guanidine extract was collected. Aβ and apoE quantification was performed by ELISAs that have been described previously

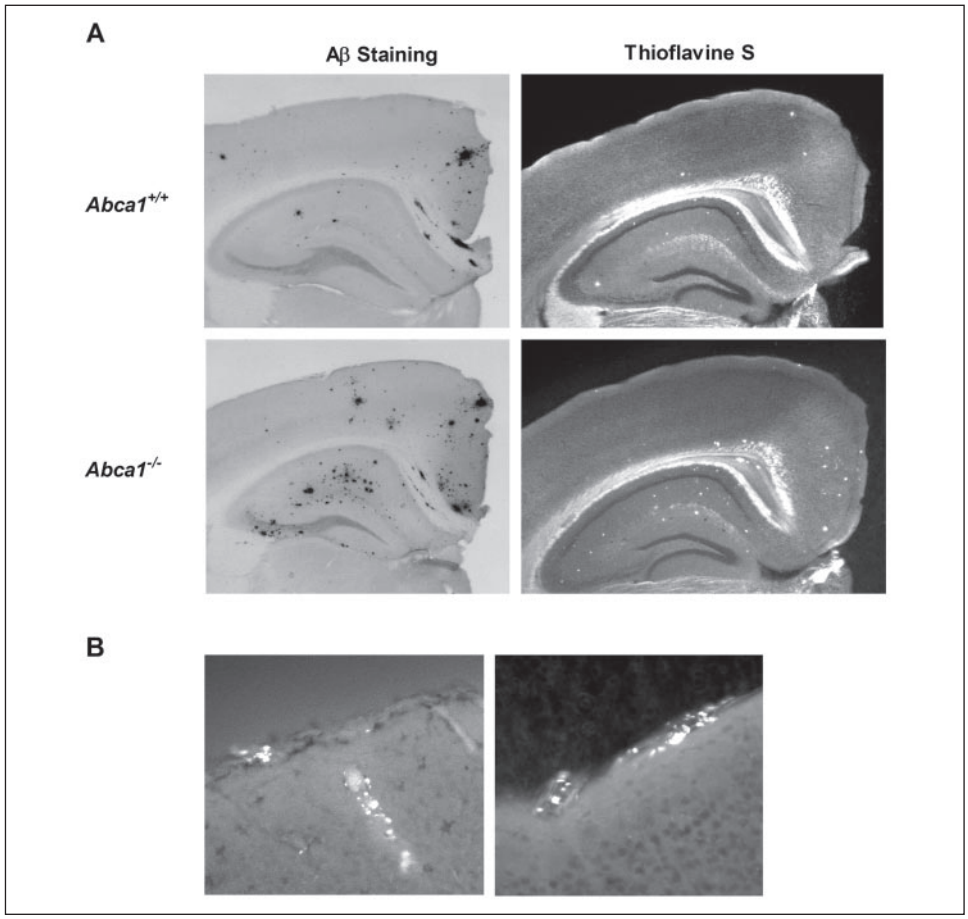

FIGURE 2. **Images of Aβ and amyloid pathology in PDAPP *Abca1* mice.** *A*, brain sections from PDAPP *Abca1* mice were immunostained with an anti-Aβ antibody to show Aβ deposition or thioflavine S to show amyloid plaques. The images represent approximately average levels of anti-Aβ immunoreactivity and thioflavine S-positive plaques in the PDAPP *Abca1*$^{+/+}$ and PDAPP *Abca1*$^{-/-}$ mice. *B*, images show examples of CAA observed in PDAPP *Abca1*$^{-/-}$ mice stained with thioflavine S.

(28, 32). Levels of Aβ and apoE in all hippocampal samples were normalized to total protein, which was determined by BCA assay (Pierce).

Histology—Frozen hemibrains from the 12-month-old mice were cut in 50-μm coronal sections from the genu of the corpus collosum to the caudal end of the hippocampus by using a sliding microtome. Sections were incubated with 3D6, an anti-Aβ monoclonal antibody, to detect Aβ deposits, and immunohistochemistry was performed as described previously (33). Thioflavine S was used to stain sections for the sub-set of Aβ that was in a β-pleated sheet (amyloid) conformation as described previously (15, 33). To examine whether apoE and amyloid were co-localized, slides were incubated with an anti-apoE antibody (Calbiochem) and then stained with thioflavine S. The area of the cingulate cortex and hippocampus covered by Aβ immunoreactivity and thioflavine S staining in sections 19, 25, and 31 (from rostral to caudal) were quantified by using stereological techniques (area fraction fractionator) as described previously (33).

Western Blots—Cortices were sonicated in 10 μl/mg RIPA buffer with protease inhibitors, and the homogenate was spun at 20,000 × *g* for 25 min. The supernatant was collected, and total protein levels were measured by BCA assay (Pierce). 15 μg of total protein was loaded per lane. Samples were run on 4–12% BisTris gels with MES running buffer (Invitrogen). Following electrophoresis, proteins were transferred to nitrocellulose membranes, which were then blocked in 4% milk in phosphate-buffered saline and probed with an antibody to the C-terminal 22 amino acids of APP (Invitrogen). For a loading control, the blots were stripped and re-probed with an anti-tubulin antibody (Sigma). Densitometric analyses used the Kodak 1D Image Analysis software.

Statistical Analysis—All analyses were performed using PRISM version 3.00 (Graphpad, San Diego). Error bars in figures represent the means ± S.E. For all tests of significance between genotypes, an analysis of variance was performed followed by Tukey's post hoc repeated measures testing between all groups. The *p* values listed are the Tukey's post hoc result. Values not listed are not significant. For testing the significance of CAA frequency in the three genotypes, a 2 degrees of freedom χ^2 test was performed.

RESULTS

Aβ Levels in CSF and Hippocampus—Levels of $A\beta_{40}$ and $A\beta_{42}$ in the CSF and hippocampi of 3- and 12-month-old PDAPP *Abca1*$^{+/+}$, *Abca1*$^{+/-}$, and *Abca1*$^{-/-}$ mice were measured using a highly sensitive ELISA. Levels of Aβ in the CSF of the mice did not vary significantly by *Abca1* genotype in either 3- or 12-month-old PDAPP mice (data not shown).

Previous studies have shown that multiple pools of Aβ exist in the brain that can be differentiated via serial extraction of the tissue in various buffers. We chose to perform carbonate extraction of the brain tissue followed by re-extraction with 5 M guanidine because similar methods have been used in publications relevant to the current study (31–34). The carbonate-soluble Aβ probably represents Aβ that is normally soluble *in vivo* or is loosely associated with membranes. Aβ that requires 5 M guanidine for extraction is likely more strongly bound to membranes or, in the case of the 12-month-old PDAPP mice, deposited into relatively insoluble amyloid plaques.

At 3 months of age, PDAPP *Abca1*$^{-/-}$ mice had significantly higher levels of carbonate-soluble $A\beta_{40}$ than PDAPP *Abca1*$^{+/+}$ mice (Fig. 1*A*), but levels of carbonate-soluble $A\beta_{42}$ and carbonate-insoluble $A\beta_{40}$ and $A\beta_{42}$ did not vary by *Abca1* genotype (Fig. 1, *A* and *B*). To investigate whether this increase of carbonate-soluble $A\beta_{40}$ in PDAPP *Abca1*$^{-/-}$

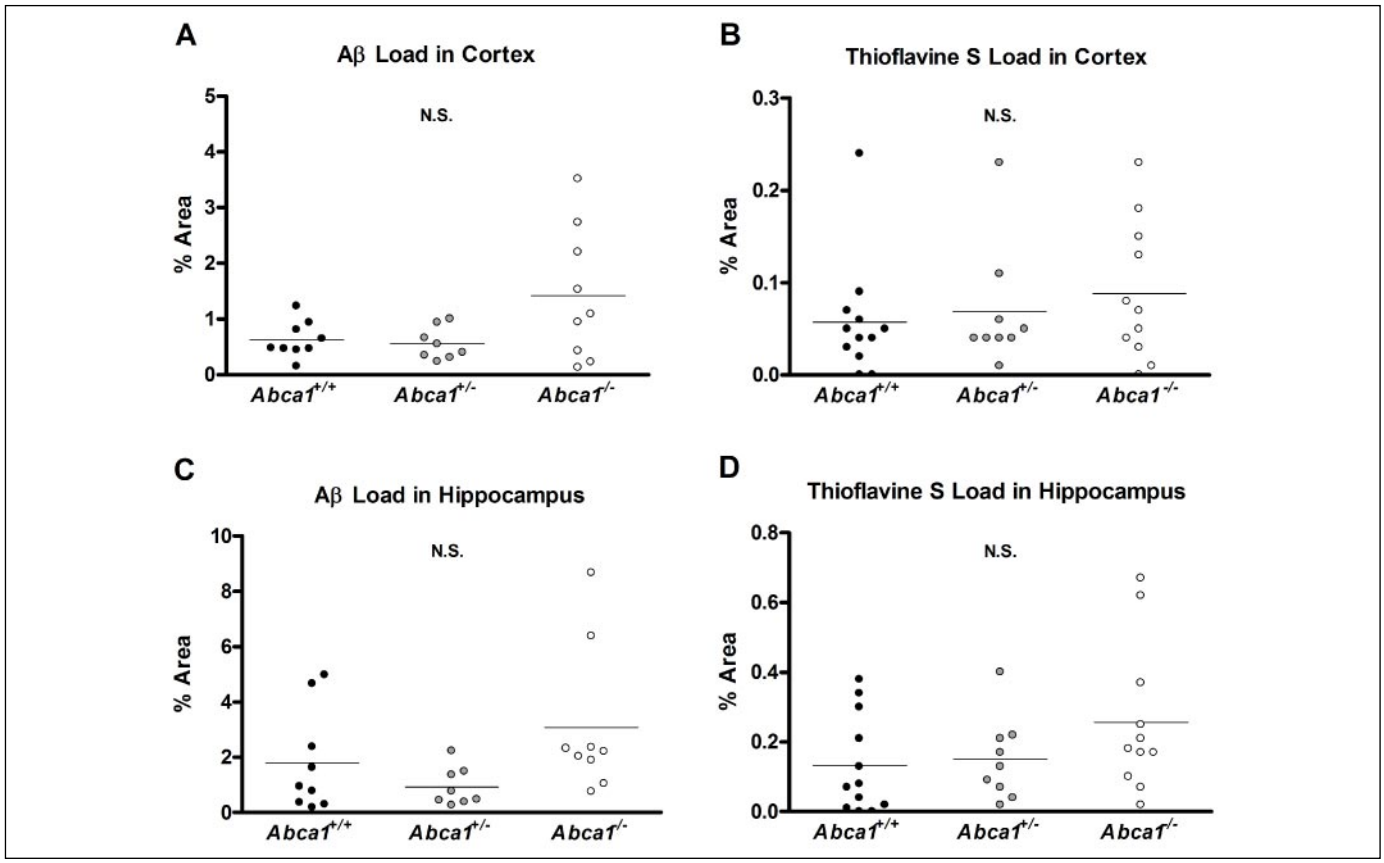

FIGURE 3. **Stereological analysis of Aβ immunoreactivity and thioflavine S-positive amyloid in 12-month-old PDAPP *Abca1* mice.** *A–D*, the graphs represent the area of cortex and hippocampus covered by Aβ immunoreactivity and thioflavine S-positive amyloid, referred to as Aβ and thioflavine S load. In the 12-month-old group, $n = 12$ for PDAPP *Abca1*$^{+/+}$, $n = 9$ for PDAPP *Abca1*$^{+/-}$, and $n = 11$ for PDAPP *Abca1*$^{-/-}$ mice. *N.S.*, not significant.

mice was a result of increased Aβ generation, we examined levels of APP and C-terminal fragments of APP (APP-CTFs) that are produced during the process of Aβ generation. We found that levels of APP and APP-CTFs, including CTF-γ, were not significantly different in 3-month-old PDAPP *Abca1*$^{+/+}$ and PDAPP *Abca1*$^{-/-}$ mice (Fig. 1*E* and data not shown). This suggests that the Aβ generation is not affected by the *Abca1* genotype. Instead, the increase of carbonate-soluble $A\beta_{40}$ in 3-month-old PDAPP *Abca1*$^{-/-}$ mice may be because of decreased clearance of $A\beta_{40}$ by poorly lipidated apoE. In fact, recent *in vivo* data suggest that apoE plays a role in $A\beta_{40}$ transport and clearance (31, 35).

At 12 months of age, Aβ deposition had begun to occur in PDAPP mice of all genotypes. The amount of carbonate-soluble Aβ increased ~10-fold in 12-month-old mice as compared with 3-month-old mice but did not vary by *Abca1* genotype (Fig. 1*C*). Moreover, there was a >100-fold increase in carbonate-insoluble $A\beta_{42}$ levels that was because of the deposition of large amounts of $A\beta_{42}$ in amyloid plaques, which require extraction in 5 M guanidine (Fig. 1*D*). Most interestingly, at this time point PDAPP *Abca1*$^{-/-}$ mice had >3-fold higher levels of $A\beta_{40}$ and $A\beta_{42}$ than PDAPP *Abca1*$^{+/+}$ mice (Fig. 1*D*, note changes in units and scales). Additionally, the average percentage of total Aβ that was carbonate-insoluble was significantly higher in PDAPP *Abca1*$^{-/-}$ mice (92% in *Abca1*$^{+/+}$ mice and 98% in *Abca1*$^{-/-}$ mice, $p < 0.001$). This suggests that more of the Aβ deposits in *Abca1*$^{-/-}$ mice were contained within insoluble plaques, potentially as a result of increased Aβ fibrillogenesis caused by poorly lipidated apoE.

Histological Analysis of Brains—Brain sections from the 12-month-old mice were immunostained for total Aβ and stained with thioflavine S for detection of fibrillar Aβ in amyloid plaques. PDAPP *Abca1*$^{-/-}$ mice had a higher average percentage of their cortex and hippocampus covered by Aβ immunoreactive and thioflavine S-positive deposits (Fig. 2*A*). However, stereological quantification of the Aβ and thioflavine S-positive plaque load did not show a significant difference between genotypes (Fig. 3). Brains were also examined for the presence of CAA with thioflavine S staining (Fig. 2*B*). Of 12 PDAPP *Abca1*$^{+/+}$ mice, none had observable CAA, whereas 1 of 9 PDAPP *Abca1*$^{+/-}$ and 4 of 11 PDAPP *Abca1*$^{-/-}$ mice had observable CAA ($p = 0.05$).

ApoE Levels in Brain—Levels of apoE in the hippocampi of the mice were measured by ELISA. As expected, the 3-month-old PDAPP *Abca1*$^{-/-}$ mice had markedly decreased apoE levels, which were ~25% the level found in PDAPP *Abca1*$^{+/+}$ mice (Fig. 4*A*). Most interestingly, even prior to plaque formation and despite the fact that *Abca1*$^{-/-}$ mice have much lower levels of total tissue apoE, *Abca1*$^{-/-}$ mice had a higher percentage of apoE in the carbonate-insoluble fraction from the hippocampus (Fig. 4*B*). This suggests that poorly lipidated apoE is less soluble *in vivo*.

After plaque deposition began, tissue-associated apoE increased in PDAPP mice of all *Abca1* genotypes and became less soluble. In 12-month-old PDAPP mice, ~40% of the apoE was not soluble in carbonate buffer and required 5 M guanidine for extraction (Fig. 4*B*), which is characteristic of proteins in amyloid plaques. More importantly, 12-month-old PDAPP mice of all *Abca1* genotypes had approximately equal levels of total apoE, which indicates that PDAPP *Abca1*$^{-/-}$ mice accumulate large amounts of apoE between 3 and 12 months of age. Additionally, the apoE accumulated by the 12-month-old PDAPP *Abca1*$^{-/-}$ mice contained a higher percentage of carbonate-insoluble apoE than found in the PDAPP *Abca1*$^{+/+}$ and PDAPP *Abca1*$^{+/-}$ mice.

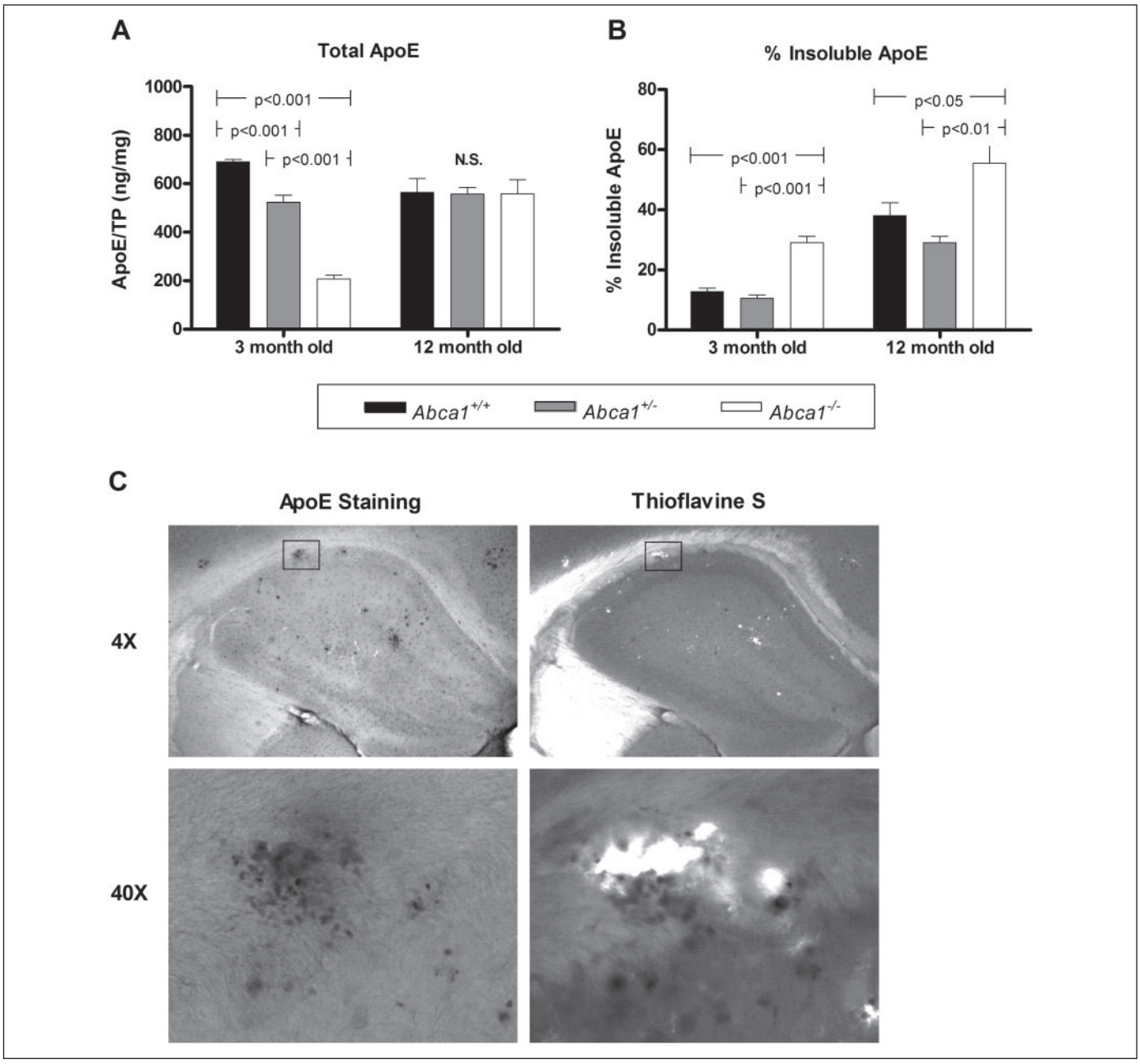

FIGURE 4. **Analysis of apoE in PDAPP mice.** *A*, apoE levels in hippocampi were measured by ELISA and normalized to total protein. *B*, the percent of insoluble apoE was calculated as the amount of apoE requiring 5 M guanidine for extraction divided by the amount of apoE soluble in both carbonate buffer and guanidine buffer. In the 3-month-old group, $n = 8$ for PDAPP *Abca1*$^{+/+}$, $n = 11$ for PDAPP *Abca1*$^{+/-}$, and $n = 13$ for PDAPP *Abca1*$^{-/-}$ mice. In the 12-month-old group, $n = 12$ for PDAPP *Abca1*$^{+/+}$, $n = 9$ for PDAPP *Abca1*$^{+/-}$, and $n = 11$ for PDAPP *Abca1*$^{-/-}$ mice. *C*, images show staining of a brain section from a 12-month-old PDAPP *Abca1*$^{-/-}$ mouse for apoE visualized by bright field microscopy (*left panels*) and for both apoE and thioflavine S visualized by fluorescence microscopy (*right panels*). The appearance of the double-staining was very similar in PDAPP *Abca1*$^{+/+}$ and PDAPP *Abca1*$^{-/-}$ mice.

In vivo, apoE normally co-deposits with Aβ into plaques. To confirm that apoE from both PDAPP *Abca1*$^{+/+}$ and PDAPP *Abca1*$^{-/-}$ mice was present in plaques, sections of brain were double-stained with anti-apoE and thioflavine S. The apoE deposits co-localized with the thioflavine S staining in both the PDAPP *Abca1*$^{+/+}$ and PDAPP *Abca1*$^{-/-}$ mice, showing that apoE was associated with amyloid plaques (Fig. 4*C*). These findings show that despite the fact that PDAPP *Abca1*$^{-/-}$ mice initially have much lower levels of apoE, the apoE that is present efficiently binds to and becomes associated with the deposited Aβ. Together, our findings suggest that the poorly lipidated apoE in PDAPP *Abca1*$^{-/-}$ mice promotes Aβ fibrillogenesis to a greater extent than normally lipidated apoE-containing HDL in the brain.

DISCUSSION

Despite initially having much lower levels of apoE in the brain, PDAPP *Abca1*$^{-/-}$ mice developed increased Aβ levels and CAA in the brain. Further supporting our findings, two other groups have observed a similar phenotype using completely independent lines of APP transgenic mice with different APP mutations and different promoters (48, 49). Although the increases in Aβ levels and CAA were significant but not dramatic in PDAPP *Abca1*$^{-/-}$ *versus* PDAPP *Abca1*$^{+/+}$ mice at 12 months of age, these results were counter to what we expected because previous studies showed an ~50% decrease in apoE levels in *Apoe*$^{+/-}$ mice resulted in a >50% decrease in Aβ levels and amyloid deposition in

both PDAPP *Apoe*$^{+/-}$ and Tg2576 *Apoe*$^{+/-}$ mice (14, 17). The demonstration that PDAPP *Abca1*$^{-/-}$ mice, which have ~25% of normal apoE levels at 3 months of age, develop increased Aβ deposition by 12 months of age suggests that poorly lipidated apoE formed in the absence of ABCA1 strongly promotes Aβ fibrillogenesis in an age-dependent manner relative to normally lipidated murine apoE.

We considered three possible mechanisms by which *Abca1* deletion could affect Aβ levels. First, we hypothesized that because ABCA1 exports cholesterol and phospholipids from cells and alterations of cellular lipids have been shown to modulate APP processing (36), it was possible that *Abca1* deletion could have modified brain Aβ levels via the effects on production of Aβ from APP. However, we found that *Abca1* deletion had no effect on levels of APP or APP-CTFs prior to Aβ deposition, which suggests that ABCA1 is not influencing Aβ production. Additionally, in our previous work we found no differences in brain total cholesterol or brain neutral lipid distribution between *Abca1*$^{+/+}$ and *Abca1*$^{-/-}$ mice (28). It is conceivable that lipid levels could be altered in certain subpopulations of cells (astrocytes or microglia) within the brain that could influence Aβ metabolism. This needs to be assessed in future experiments. A second possible mechanism by which *Abca1* deletion could increase Aβ deposition is that the lipid-poor apoE in *Abca1*$^{-/-}$ mice may impair receptor-mediated clearance of Aβ. This hypothesis is based on data showing that lipid-poor apoE is a poor ligand for the low density lipoprotein receptor (LDLR) and LDLR-related protein, the major apoE receptors in brain (37–39). Previous data from our laboratory have shown increased soluble Aβ in the brains of 3-month-old PDAPP mice lacking apoE, which is possibly due to a lack of receptor-mediated clearance of apoE-Aβ complexes (31). In the current study, we found increased soluble $A\beta_{40}$ in the brains of PDAPP *Abca1*$^{-/-}$ mice, also possibly a result of impaired receptor-mediated clearance of apoE-Aβ complexes. However, PDAPP mice lacking apoE, and therefore lacking receptor-mediated clearance of apoE-Aβ complexes, have decreased Aβ deposition, whereas PDAPP *Abca1*$^{-/-}$ mice have increased Aβ deposition. This strongly suggests that receptor-mediated clearance of Aβ is not the main reason for the increase in Aβ we observed in 12-month-old PDAPP *Abca1*$^{-/-}$ mice. Finally, we think the most likely mechanism by which *Abca1* deletion increases Aβ deposition is by affecting the lipidation state of apoE. Experiments have shown that apoE lipidation affects interactions with Aβ *in vitro* that is likely to influence the probability that Aβ will aggregate. De-lipidated apoE3 and apoE4 form similar amounts of SDS-stable complexes with Aβ (2, 40). In contrast, cell-secreted, lipidated apoE2 and apoE3 interact with Aβ and form a much greater amount of SDS-stable complex than apoE4 (41, 42). Furthermore, the affinity of lipidated *versus* nonlipidated apoE isoforms for soluble Aβ is significantly higher (11). Although the effect of altering the relative amount of apoE lipidation on Aβ clearance and fibrillogenesis has not been assessed *in vitro*, these previous findings suggest that lipidation state of apoE can markedly influence its interactions with Aβ.

Recent data using liver X receptor (LXR) agonists both *in vitro* (43–46) and *in vivo* (46) have shown that they can affect Aβ levels. LXR dimerizes with retinoid X receptor to transcriptionally induce a group of lipid-related genes including *Abca1*. It was recently shown that the LXR agonist T0901317 decreased brain $A\beta_{40}$ and $A\beta_{42}$ levels when given over several days to 3-month-old APP transgenic mice (APP23) prior to plaque deposition (46). One possible mechanism for this effect is that the LXR agonist modulated cellular cholesterol levels and directly affected APP processing into Aβ (36). Although the authors found a difference in secreted APP fragments, they did not see any differences in APP-CTFs that would support this mechanism. An alternative hypothesis to explain these data is that the induction of ABCA1 increases lipidation of apoE, which could affect Aβ levels and ultimately Aβ deposition.

Because a decrease in ABCA1 results in more amyloid deposition, increasing ABCA1 protein or function might be predicted to decrease amyloid deposition via increasing apoE lipidation. This hypothesis needs to be tested directly. If ABCA1 influences amyloid deposition by altering the level and lipidation state of apoE, it will be important to assess the effects of ABCA1 on both murine and human apoE. This is because murine apoE appears to increase amyloid deposition, whereas human apoE appears to delay and decrease amyloid deposition (34, 35, 47).

In sum, the absence of ABCA1 resulted in an increase in amyloid deposition and CAA in PDAPP mice. This effect appears likely because of promotion of Aβ fibrillogenesis by the poorly lipidated apoE particles produced in the brains of *Abca1*$^{-/-}$ mice. These results emphasize the potential importance of ABCA1 not only in regulating apoE levels and lipidation but also the consequences of its absence on Aβ deposition and conformation. As such, ABCA1 can be hypothesized to be a potential therapeutic target in AD, and this hypothesis can be tested in future studies.

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