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 Abca1 /−/− 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) 3 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 modulating 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 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 into 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 apoA1 and apoAII (25, 26). Abca1 /−/− mice have a similar phenotype as patients with Tangier disease, with greatly decreased HDL and apoA1 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-
lsterol/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-Abca1tm1Idm). 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 carbonatesoluble supernatant was collected, and the pellet was re-homogenized with 700 μl of guanidine buffer (5 mM 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.
Brain Aβ Deposition in PDAPP Abca1<sup>−/−</sup> Mice

**FIGURE 2. Images of Aβ and amyloid pathology in PDAPP Abca1<sup>−/−</sup> mice.** A, brain sections from PDAPP Abca1<sup>−/−</sup> 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<sup>−/−</sup> and PDAPP Abca1<sup>+/+</sup> mice. B, images show examples of CAA observed in PDAPP Abca1<sup>−/−</sup> 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 callosum 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 colocalized, 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<sup>2</sup> test was performed.

**RESULTS**

**Aβ Levels in CSF and Hippocampus**—Levels of Aβ<sub>40</sub> and Aβ<sub>42</sub> in the CSF and hippocampi of 3- and 12-month-old PDAPP Abca1<sup>+/+</sup>, Abca1<sup>+/−</sup>, and Abca1<sup>−/−</sup> 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<sup>−/−</sup> mice had significantly higher levels of carbonate-soluble Aβ<sub>40</sub> than PDAPP Abca1<sup>+/+</sup> mice (Fig. 1A), but levels of carbonate-soluble Aβ<sub>42</sub> and carbonate-insoluble Aβ<sub>40</sub> and Aβ<sub>42</sub> did not vary by Abca1 genotype (Fig. 1, A and B). To investigate whether this increase of carbonate-soluble Aβ<sub>40</sub> in PDAPP Abca1<sup>−/−</sup>...
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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. 1E and data not shown). This suggests that the Aβ generation is not affected by the Abca1 genotype. Instead, the increase of carbonate-soluble Aβ1−40 in 3-month-old PDAPP Abca1−/− mice may be because of decreased clearance of Aβ1−40 by poorly lipidated apoE. In fact, recent in vivo data suggest that apoE plays a role in Aβ 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. 1C). Moreover, there was a >100-fold increase in carbonate-insoluble Aβ1−42 levels that was because of the deposition of large amounts of Aβ1−42 in amyloid plaques, which require extraction in 5 M guanidine (Fig. 1D). Most interestingly, at this time point PDAPP Abca1−/− mice had >3-fold higher levels of Aβ1−40 and Aβ1−42 than PDAPP Abca1+/+ mice (Fig. 1D). 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 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.

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. 4B), 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.
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. 4C). 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<sup>+/−</sup> and Tg2576 Apoe<sup>+/−</sup> mice (14, 17). The demonstration that PDAPP Abca1<sup>−/−/−</sup> 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<sup>−/−</sup> and Abca1<sup>+/−</sup> 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<sup>−/−</sup> 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 Aβ<sub>40</sub> in the brains of PDAPP Abca1<sup>−/−/−</sup> 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<sup>−/−/−</sup> 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<sup>−/−/−</sup> 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<sub>40</sub> 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 Aβ<sub>40</sub> and Aβ<sub>42</sub> 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 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<sup>−/−/−</sup> 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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