Physiologically regulated transgenic ABCA1 does not reduce amyloid burden or amyloid-β peptide levels in vivo

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Abstract ABCA1-deficient mice have low levels of poorly lipidated apolipoprotein E (apoE) and exhibit increased amyloid load. To test whether excess ABCA1 protects from amyloid deposition, we crossed APP/PS1 mice to *ABCA1* bacterial artificial chromosome (BAC) transgenic mice. Compared with wild-type animals, the *ABCA1* BAC led to a 50% increase in cortical ABCA1 protein and a 15% increase in apoE abundance, demonstrating that this BAC supports modest ABCA1 overexpression in brain. However, this was observed only in animals that do not deposit amyloid. Comparison of ABCA1/APP/PS1 mice with APP/PS1 controls revealed no differences in levels of brain ABCA1 protein, amyloid, Aβ, or apoE, despite clear retention of ABCA1 overexpression in the livers of these animals. To further investigate ABCA1 expression in the amyloid-containing brain, we then compared ABCA1 mRNA and protein levels in young and aged cortex and cerebellum of APP/PS1 and ABCA1/APP/PS1 animals. Compared with APP/PS1 controls, aged ABCA1/APP/PS1 mice exhibited increased ABCA1 mRNA, but not protein, selectively in cortex. Additionally, ABCA1 mRNA levels were not increased before amyloid deposition but were induced only in the presence of extensive Aβ and amyloid levels. These data suggest that an induction of ABCA1 expression may be associated with late-stage Alzheimer's neuropathology.—Hirsch-Reinshagen, V., J. Y. Chan, A. Wilkinson, T. Tanaka, J. Fan, G. Ou, L. F. Maia, R. R. Singaraja, M. R. Hayden, and C. L. Wellington. **Physiologically regulated transgenic ABCA1 does not reduce amyloid burden or Aβ peptide levels in vivo. *J. Lipid Res.* 2007.** 48: **914–923.**

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Apolipoprotein E (apoE) is a well-validated risk factor for late-onset Alzheimer's disease (AD) (1). In the central nervous system (CNS), apoE is secreted by astrocytes and microglia and serves as the major cholesterol carrier in brain (2). ApoE also binds amyloid-β peptide (Aβ) and is found in amyloid plaques (3, 4). ApoE-deficient mice deposit Aβ but not amyloid, (5, 6), and the extent of amyloid deposition correlates with apoE gene dose (7). These findings suggest that factors that regulate apoE abundance may affect amyloidogenesis.

We and others have shown that ABCA1 modulates CNS apoE levels (8–10). ABCA1 effluxes cellular lipids onto lipid-poor apolipoprotein acceptors (11), and deficiency of ABCA1 results in nearly undetectable plasma HDL levels, impaired cholesterol efflux, and an increased risk of cardiovascular disease (12–14). In the brain, ABCA1 is expressed in neurons, astrocytes, and microglia and is induced by liver X receptor (LXR) and retinoic X receptor (RXR) agonists (15–18). ABCA1-deficient glia secrete less apoE than wild-type cells and are impaired in cholesterol efflux to apoE (8). These effects may underlie the drastic reduction in CNS apoE levels found in ABCA1-deficient mice and explain the poor lipidation of the remaining apoE particles (8–10).

Recently, we and others demonstrated that ABCA1 also modulates amyloid burden in vivo (19–21). Because apoE levels correlate with the extent of amyloid load, the reduced levels of apoE in ABCA1-deficient mice led to the prediction that fewer amyloid deposits would be observed in the absence of ABCA1. In contrast, ABCA1-deficient mice developed an equal or greater amyloid burden than wild-type mice in four independent models of AD (19–21), demonstrating that poorly lipidated apoE markedly promotes amyloidogenesis. These observations raise the converse possibility that selective overexpression of ABCA1 may enhance apoE lipidation and lead to reduced amyloid levels.

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Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; apoE, apolipoprotein E; BAC, bacterial artificial chromosome; CNS, central nervous system; CSF, cerebrospinal fluid; LXR, liver X receptor; RXR, retinoic X receptor; Tg, transgenic; 24S-OH-Chol, 24*S*-hydroxycholesterol; 27-OH-Chol, 27-hydroxycholesterol.

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To test this possibility, we crossed the APP/PS1 mouse model of AD to *ABCA1* bacterial artificial chromosome (BAC) transgenic (Tg) mice, which express human *ABCA1* from endogenous regulatory signals (22). BAC Tg models are desirable for many in vivo investigations, as the transgene is expressed from physiologically relevant regulatory elements that conserve appropriate developmental and tissue-specific expression patterns (23). Accordingly, expression of human *ABCA1* from a BAC transgene has been shown to mirror that of endogenous murine *abca1* in multiple tissues, including brain (24), and protects from atherosclerosis in both whole animal and bone marrow transplant paradigms (25, 26).

Here, we report that although the *ABCA1* BAC leads to modestly increased ABCA1 protein levels in brain, this is observed only in animals without amyloid deposits. In animals with advanced AD neuropathology, ABCA1 protein levels are not increased by the presence of the *ABCA1* BAC, even though human and murine ABCA1 mRNA levels are both increased. Accordingly, no changes in amyloid, apoE, or Aβ levels are found between APP/PS1 mice in the absence or presence of human *ABCA1*. These observations suggest that the expression of ABCA1 may be subject to transcriptional and posttranscriptional control in brain with advanced AD, which ultimately may attenuate ABCA1 protein accumulation in vivo.

MATERIALS AND METHODS

Animals

ABCA1 BAC Tg mice containing BAC RP11-32H03 have been described previously (27) and are maintained on a congenic C57Bl/6 genetic background. APP/PS1 (line 85) mice (Jackson Laboratories, Bar Harbor, ME) express a chimeric mouse/human APP650 cDNA containing the Swedish (KM670/671NL) mutation cointegrated with the human presenilin 1 (PS1) gene containing the ΔE9 mutation (28) and are maintained on an F1 50% C3H, 50% C57Bl/6 genetic background. APP/PS1 mice were crossed to *ABCA1* BAC Tg animals, and double ABCA1/APP/PS1 Tg animals were compared with APP/PS1 littermate controls, all with a 75% C57Bl/6, 25% C3H genetic composition. Animals were maintained on a chow diet (PMI LabDiet 5010), and all animal procedures were in accordance with the Canadian Council of Animal Care and the University of British Columbia Committee on Animal Care.

Protein extraction and Western blot

Murine tissue extractions were performed as described previously (19). Briefly, brain regions were homogenized in ice-cold PBS containing Complete protease inhibitor (Roche, Mississauga, Canada) in a Tissuemite homogenizer followed by centrifugation. The supernatant (soluble fraction) was then removed and used to evaluate soluble apoE. The pellets from the PBS solubilization step were resuspended in ice-cold lysis buffer containing 10% glycerol, 1% Triton X-100, and Complete protease inhibitor (Roche) in PBS and centrifuged to extract ABCA1. The pellet from this step (insoluble fraction) was finally solubilized in 5 M guanidine hydrochloride in 50 mM Tris-HCl, pH 8.0, to evaluate plaque-associated Aβ. Brain tissues from all animals were extracted in an identical manner, and all fractions were immediately frozen at −80°C until analysis. Liver tissues were homogenized directly into ice-cold lysis buffer containing 10% glycerol, 1% Triton X-100, and Complete protease inhibitor (Roche) in PBS. Protein concentrations were determined by DC Protein Assay (Bio-Rad, Hercules, CA). For Western blots, tissue lysates were resolved by SDS-PAGE and immunodetected using a monoclonal anti-ABCA1 antibody (AC10) (16) or an anti-GAPDH antibody (Chemicon, Temecula, CA) as a loading control. Blots were developed using enhanced chemiluminescence (Amersham, Piscataway, NJ) and quantified using NIH Image J software.

ApoE ELISA

Murine apoE levels were determined by ELISA as described previously (9). Plates were coated with anti-apoE (WU E-4) (a kind gift from D. Holtzman, Washington University). Samples were diluted in 0.5% BSA and 0.025% Tween-20 in PBS. Standards were based on plasma from Swiss-Webster rats containing 61.7 μg/ml apoE (9). After an overnight incubation at 4°C, plates were incubated with goat anti-apoE (EMD Biosciences, San Diego, CA) followed by biotinylated anti-goat antibody (Vector Laboratories, Burlington, CA). Plates were developed with poly-horseradish peroxidase streptavidin (Pierce, Rockford, IL) and ultra-slow 3,3′,5,5′-tetramethylbenzidine (Sigma, St. Louis, MO), stopped with 1 N HCl, and read at 450 nm.

Cerebrospinal fluid cholesterol measurements

Cerebrospinal fluid (CSF) was isolated as described previously (29). Total cholesterol measurements were performed using the fluorogenic Amplex Red Cholesterol Assay Kit (Molecular Probes, Burlington, CA).

Neuropathological analyses

Thioflavine-S staining of amyloid plaques was performed and quantified as described (19). Human Aβ levels were quantified by ELISA (Biosource, Camarillo, CA) and normalized to total protein.

Quantitative RT-PCR

RNA was extracted using Trizol (Invitrogen, Burlington, CA) and treated with DNaseI. cDNA was generated using oligo-dT primers and Taqman reverse transcription reagents (Applied Biosystems, Foster City, CA). Quantitative real-time PCR primers were designed using PrimerExpress (Applied Biosystems) to span human- and murine-specific regions of ABCA1 or regions identical in both species. Primer sequences and cycling conditions will be provided upon request. Real-time quantitative PCR was done with SYBR Green reagents (Applied Biosystems) on an ABI 7000 (Applied Biosystems). Each sample was assayed at least in duplicate, normalized to β-actin, and analyzed with 7000 system SDS software version 1.2 (Applied Biosystems) using the relative standard curve method.

Statistical analysis

Data are shown as means ± SEM and were analyzed by two-tailed unpaired Student's *t*-tests for comparisons between two groups or by one-way ANOVA with a Newman-Keul's posttest for comparisons among multiple groups. Welch's correction for unequal variances was applied when variances were significantly different between groups. Analyses were performed using GraphPad Prism (version 4.0; GraphPad, San Diego, CA).

RESULTS

ABCA1 and apoE levels are increased in cortex of *ABCA1* BAC Tg mice

To test whether excess ABCA1 can mitigate amyloid deposition, *ABCA1* BAC Tg mice were crossed to the APP/

PS1 model of AD, resulting in four groups of animals consisting of wild type, *ABCA1* BAC, APP/PS1, and ABCA1/APP/PS1 littermates that are optimally matched for their mixed (75% C57Bl/6, 25% C3H) genetic background. To first validate the *ABCA1* BAC Tg model for this study, we confirmed that the presence of the *ABCA1* BAC transgene resulted in increased ABCA1 levels in adult brain (in animals of 14.5 ± 1.35 months of age). Compared with wild-type animals, ABCA1 protein levels were increased by ~50% in *ABCA1* BAC Tg cortex and by ~100% in liver (cortex, $P = 0.0009$, n ⩾ 13; liver, $P = 0.0042$, n = 8) (**Fig. 1A**). These values represent the sum of both human and murine ABCA1 protein, as our ABCA1 antibody does not distinguish between human and murine ABCA1. That human ABCA1 expression was considerably more robust in the liver compared with the brain suggests that the endogenous regulatory sequences present on BAC RP11-23H03 are less efficiently recognized in brain, that BAC RP11-23H03 may be missing some elements required for robust expression in cells in the CNS, or that ABCA1 may be expressed in a greater proportion of total cells in liver compared with brain.

We next determined whether increased brain ABCA1 resulted in increased cortical and CSF apoE levels and CSF cholesterol (Fig. 1B). Consistent with the modest degree of ABCA1 overexpression in the BAC Tg mice, PBS-soluble apoE levels were slightly, but significantly, increased by 15% in the cortex of *ABCA1* BAC Tg mice (mean apoE level = 185.1 ng/mg) compared with wild-type controls (mean apoE level = 160.9 ng/mg) ($P = 0.036$, n ⩾ 12). However, no significant changes were observed in either CSF apoE levels or CSF cholesterol in the presence of ABCA1 overexpression. These data show that physiological overexpression of ABCA1 protein in the BAC Tg model is sufficient to promote a subtle increase in apoE levels in brain tissue but is unable to alter the levels or lipidation of circulating apoE in CSF, where particles are subject to remodeling.

Amyloid burden and Aβ and apoE levels are not changed by the presence of physiologically regulated human ABCA1

To determine whether excess ABCA1 may inhibit amyloidogenesis in vivo, we then analyzed APP/PS1 mice with and without the *ABCA1* BAC at 14.5 months of age. No significant differences in amyloid burden, guanidine-extractable Aβ levels, soluble apoE levels, or CSF cholesterol were observed between ABCA1/APP/PS1 Tg animals (APP/BAC+) relative to APP/PS1 littermate controls (APP/BAC−) (**Fig. 2**). These data show that physiologically regulated expression of human ABCA1 from endogenous regulatory elements present on BAC RP11-32H03 does not significantly affect Aβ or apoE metabolism in vivo in aged APP/PS1 mice.

Human ABCA1 is expressed in brain and liver of ABCA1/APP/PS1 mice but makes a significant contribution to total ABCA1 protein levels only in liver

To determine whether the inability to detect a change in amyloid, Aβ, apoE, or CSF cholesterol in the ABCA1/APP/PS1 mice could be attributed to an unexpected loss of ABCA1 expression from the BAC, quantitative RT-PCR was used to quantify human, murine, and total ABCA1 mRNA of ABCA1/APP/PS1 (APP/BAC+) mice compared with APP/PS1 littermates (APP/BAC−). Validation tests first confirmed that the human primers were unable to amplify murine *abca1* mRNA and that the murine primers did not amplify human *ABCA1* mRNA (data not shown). As expected, human *ABCA1* mRNA was present only in

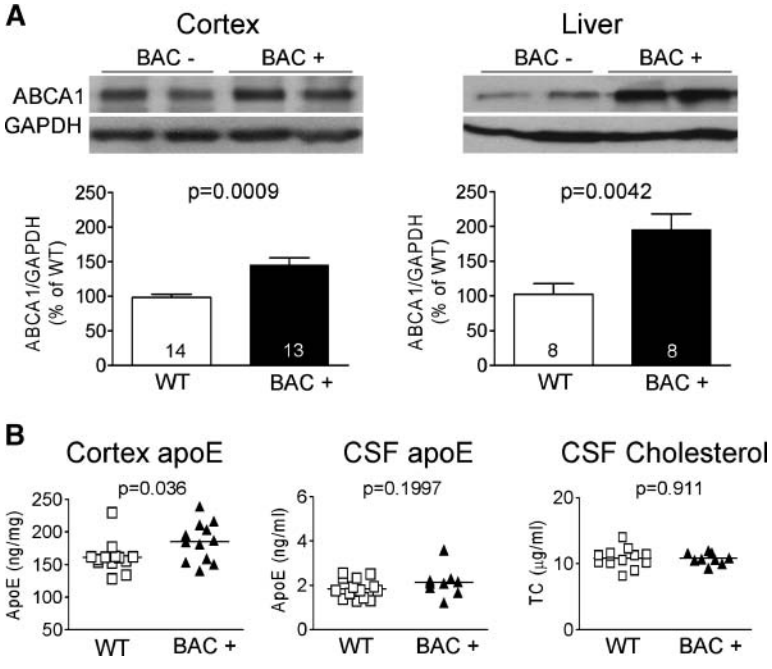

Fig. 1. The *ABCA1* bacterial artificial chromosome (BAC) increases cortical and liver ABCA1 and cortical apolipoprotein E (apoE) levels but does not affect cerebrospinal fluid (CSF) apoE or cholesterol. A: Total ABCA1 protein in cortex and liver was measured by Western blot and quantitated by densitometry relative to GAPDH levels. Graphs are expressed as percentage of wild-type levels (wild-type animals were assigned a 100% level) and illustrate the pooled results of four independent measurements of 14 wild-type (WT) and 13 *ABCA1* BAC transgenic (Tg) (BAC+) cortical samples and at least two independent measurements of 8 wild-type and 8 *ABCA1* BAC Tg liver samples. Values shown are means ± SEM. B: Left panel: PBS-soluble cortical apoE levels were measured by ELISA and normalized for total protein. The graph represents two independent experiments of 13 wild-type (WT) and 12 *ABCA1* BAC Tg (BAC+) samples measured in triplicate. Middle panel: CSF apoE levels were assessed by ELISA. The graph represents 14 wild-type (WT) and 9 *ABCA1* BAC Tg (BAC+) samples. Right panel: CSF total cholesterol was analyzed using the Amplex Red Cholesterol Assay Kit. The graph represents 13 wild-type (WT) and 9 *ABCA1* BAC Tg (BAC+) samples. Two-tailed Student's *t*-tests were used for statistical analyses.

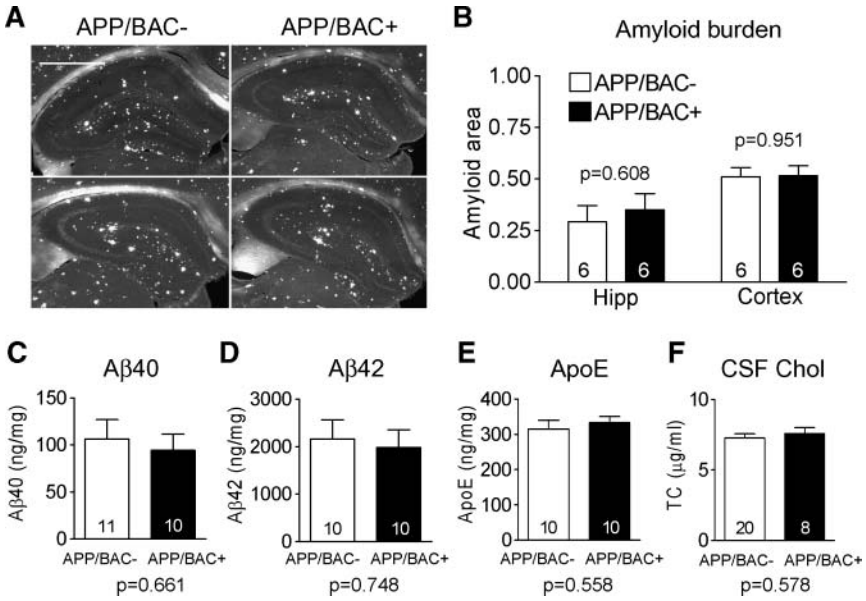

Fig. 2. *ABCA1* BAC transgene expression has no significant effect on amyloid burden, Aβ level, apoE abundance, or CSF cholesterol. A: Thioflavine-S staining of hemicoronal sections from APP/PS1 hippocampi in the presence (APP/BAC+) and absence (APP/BAC−) of the *ABCA1* BAC transgene. Images show two individual mice per genotype and correspond to 2.5× magnification of the hippocampus. Bar = 1,000 μm. Amyloid load was quantitated for hippocampus and cingulate cortex in six APP/PS1 (APP/BAC−) and six ABCA1/APP/PS1 (APP/BAC+) animals. B: The graph is expressed as the percentage of Thioflavine-S-positive area over total area. C, D: Guanidine-extractable Aβ40 (C) and Aβ42 (D) were measured by ELISA and normalized for total protein. The graphs correspond to 10 APP/PS1 (APP/BAC−) and 10 ABCA1/APP/PS1 (APP/BAC+) animals. E: PBS-soluble cortical apoE levels were measured by ELISA and normalized for total protein. The graph represents two independent measurements (each performed in triplicate) of ten APP/PS1 (APP/BAC−) and ten ABCA1/APP/PS1 (APP/BAC+) animals. F: CSF cholesterol (Chol) levels were measured by Amplex Red assay in 20 APP/PS1 (APP/BAC−) and eight ABCA1/APP/PS1 (APP/BAC+) mice. In all graphs, sample size is indicated by the number within the bar. Two-tailed Student's *t*-tests were used for all statistical analyses. Values shown are means ± SEM.

animals carrying the *ABCA1* BAC transgene (**Fig. 3A**), and murine *abca1* mRNA levels were similar in ABCA1/APP/PS1 (APP/BAC+) compared with APP/PS1 (APP/BAC−) mice, showing that the presence of the human *ABCA1* BAC transgene has no significant influence on murine *abca1* mRNA levels (Fig. 3B). Surprisingly, we observed no increase in total ABCA1 mRNA ($P > 0.05$, $n \geq 6$) or protein ($P > 0.05$, $n \geq 10$) levels in cortical extracts prepared from APP/PS1 (APP/BAC−) and ABCA1/APP/PS1 (APP/BAC+) mice (Fig. 3C–E), suggesting that the human *ABCA1* BAC makes a negligible contribution to total ABCA1 in the amyloid-containing brain.

ABCA1 expression was also evaluated in the liver of these same mice. As expected, human *ABCA1* mRNA was detected only in animals carrying the *ABCA1* BAC transgene (**Fig. 4A**), and murine *abca1* mRNA levels were no different between groups (Fig. 4B). Unlike brain, however, total ABCA1 mRNA and protein levels were increased by ~2-fold in ABCA1/APP/PS1 livers (APP/BAC+) compared with APP/PS1 controls (APP/BAC−) ($P = 0.0345$, $n \geq 5$ for mRNA, $P = 0.0003$, $n = 8$ for protein) (Fig. 4D, E). These results demonstrate that the human BAC remains functional after breeding to APP/PS1 mice but that its expression in brain may be altered in mice with amyloid deposits.

ABCA1 mRNA but not protein levels are increased in the amyloid-containing brain

To further explore these findings, we then compared *ABCA1* mRNA and protein levels in cortex and cerebellum in littermate mice of all four genotypes. Compared with wild-type mice, murine *abca1* mRNA levels were increased significantly in the cortex of APP/PS1 (APP/BAC−) ($P < 0.05$, $n = 8$) and ABCA1/APP/PS1 (APP/BAC+) ($P < 0.05$, $n = 6$) mice but were unchanged in cerebellum ($P > 0.05$, $n > 7$) (**Fig. 5A**). Similarly, human *ABCA1* mRNA levels were ~3-fold higher in cortex ($P < 0.05$, $n \geq 6$) but not in cerebellum ($P > 0.05$, $n \geq 7$) of ABCA1/APP/PS1 (APP/BAC+) compared with *ABCA1* BAC Tg (BAC+) mice (Fig. 5B). However, this increase in cortical *ABCA1* mRNA was not necessarily accompanied by a proportional increase of ABCA1 protein. Compared with wild-type mice, animals with the *ABCA1* BAC alone or with the APP/PS1 transgenes alone each displayed a very modest but significant increase of total ABCA1 protein in cortex ($P < 0.01$, $n \geq 10$, each group compared with wild-type controls) (Fig. 5C). However, compared with APP/PS1 (APP/BAC−) mice, ABCA1/APP/PS1 (APP/BAC+) mice displayed no further increase in cortical ABCA1 protein levels, despite having significantly increased murine and

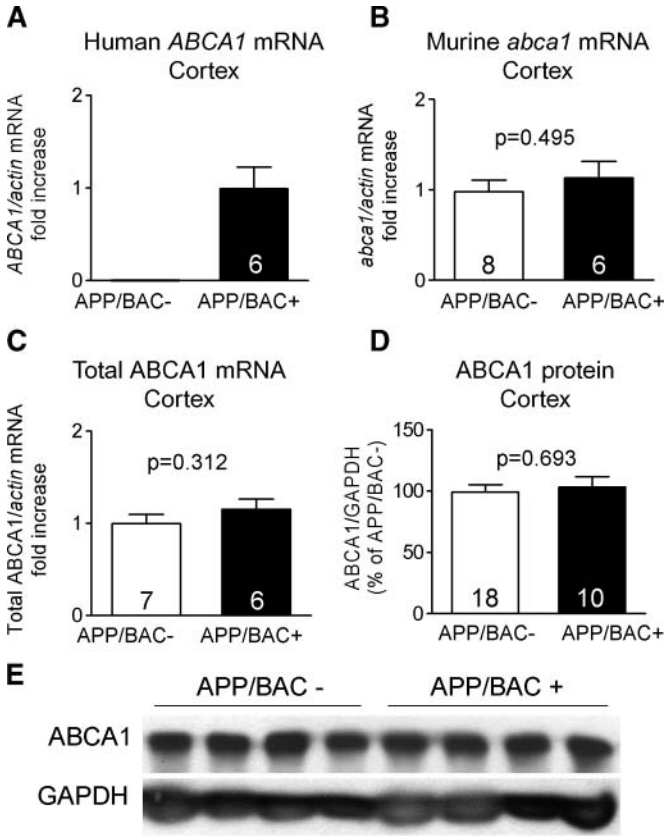

Fig. 3. Human *ABCA1* expression in the cortex of APP/PS1 mice is insufficient to increase total *ABCA1* mRNA or protein levels. A–C: *ABCA1* mRNA levels were determined using quantitative RT-PCR using human-specific primers (A), murine-specific primers (B), or primers that do not distinguish between species (C). Graphs are expressed as fold difference of ABCA1/APP/PS1 (APP/BAC+) mice relative to APP/PS1 (APP/BAC−) controls, which were assigned an arbitrary value of 1. Numbers in each bar represent the number of independent animals that were analyzed, using at least duplicate measurements per animal. D, E: ABCA1 protein levels were evaluated by Western blot and normalized to GAPDH as an internal loading control from ≥10 independent mice per genotype. Two-tailed Student's *t*-tests were used for all statistical analyses. Values shown are means ± SEM.

human *ABCA1* mRNA levels relative to wild-type controls ($P > 0.05$, n ≥ 10) (Fig. 5C). Cerebellar ABCA1 protein levels showed no significant difference in ABCA1 protein levels among groups ($P > 0.05$, n ≥ 8) (Fig. 5C).

ABCA1 expression is induced only in mice with advanced disease

The results described above showing that ABCA1 mRNA levels are increased in aged APP/PS1 mice suggest that either ABCA1 may be induced in response to Aβ or amyloid deposition or the that the presence of the APP/PS1 transgenes may alter ABCA1 expression even before amyloid neuropathology begins. To further determine the relationship between ABCA1 expression and age, we examined ABCA1 expression in cortex and cerebellum of young (2.7 ± 0.24 months) and old (12.7 ± 0.71 months) APP/PS1 mice. Because the human *ABCA1* BAC makes little contribution to total ABCA1 mRNA levels in brain, the animals used in this experiment were baseline APP/PS1 mice on an F1 50% C3H, 50% C57Bl/6 genetic background, which is slightly different from the animals analyzed above, on a 75% C57Bl/6, 25% C3H background.

As expected, murine *abca1* mRNA levels were not increased in 2.7 month old APP/PS1 cortex or cerebellum compared with wild-type controls ($P > 0.05$, n ≥ 4 for each group) (**Fig. 6A**), demonstrating that ABCA1 is not induced simply by expressing the APP and PS1 transgenes before the initiation of amyloid deposition. Also as expected, APP/PS1 mice showed no increase in cerebellar *abca1* mRNA expression even at 12.7 months of age (Fig. 6A, right panel). Unexpectedly, however, we did not observe a significant induction in cortical *abca1* mRNA levels in 12.7 month old APP/PS1 mice (Fig. 6A, left panel), in contrast with our observations in Fig. 5. No sig-

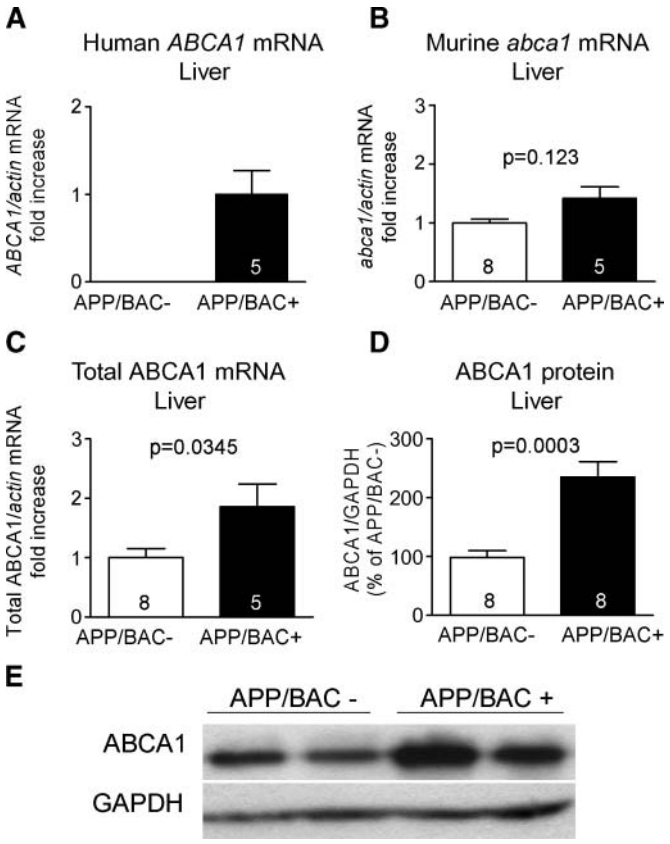

Fig. 4. Human ABCA1 expression in the liver of APP/PS1 mice results in significant increases in total *ABCA1* mRNA and protein levels. A–C: *ABCA1* mRNA levels were determined using quantitative RT-PCR using human-specific primers (A), murine-specific primers (B), or primers that do not distinguish between species (C). Graphs are expressed as fold difference of ABCA1/APP/PS1 (APP/BAC+) mice relative to APP/PS1 (APP/BAC−) controls, which were assigned an arbitrary value of 1. Numbers in each bar represent the number of independent animals that were analyzed, using at least duplicate measurements per animal. D, E: ABCA1 protein levels were evaluated by Western blot and normalized to GAPDH as an internal loading control from eight independent mice per genotype. Two-tailed Student's *t*-tests were used for all statistical analyses. Values shown are means ± SEM.

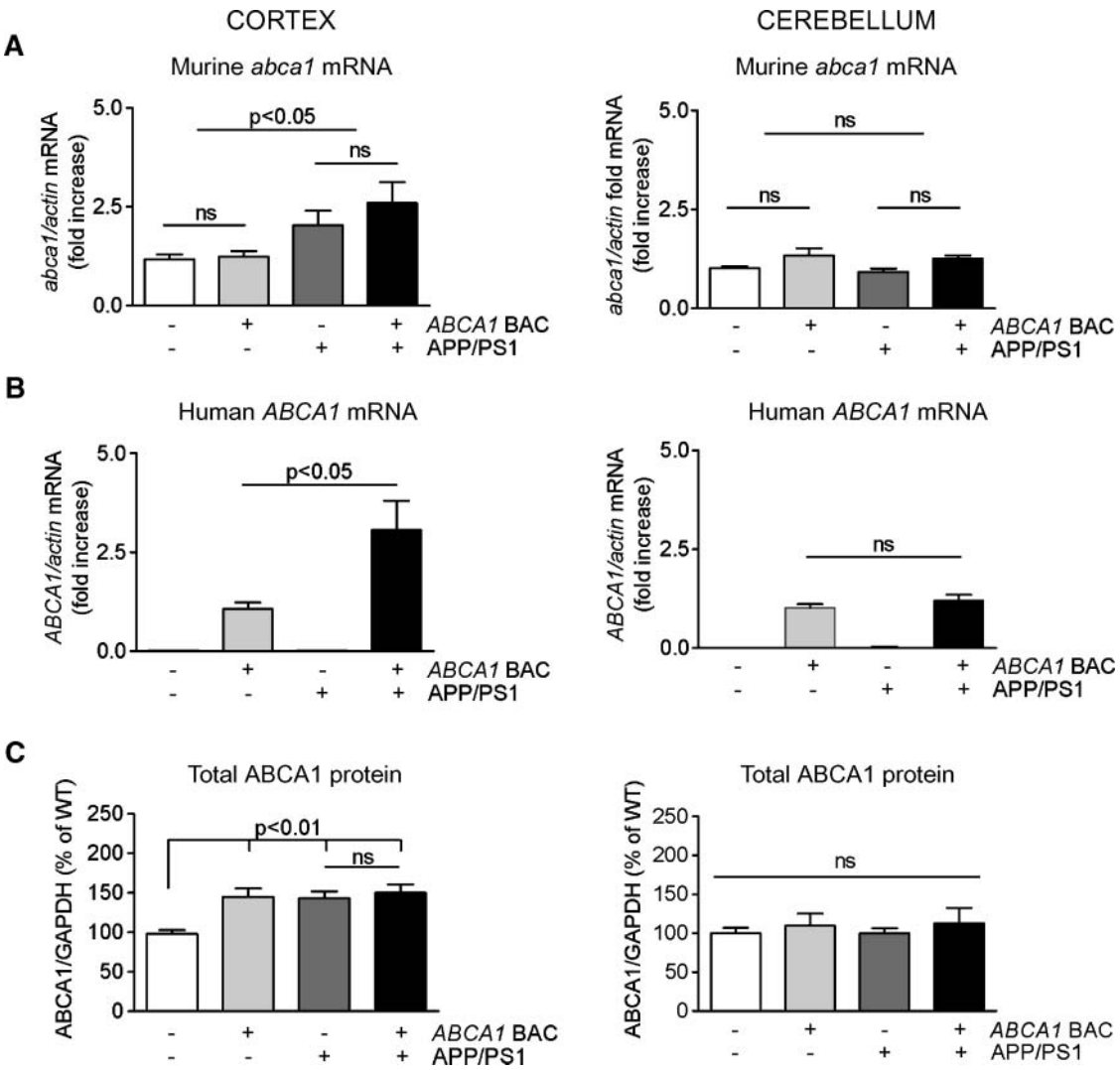

Fig. 5. Regional specificity of ABCA1 induction. A, B: Murine (A) and human (B) *ABCA1* mRNA levels were determined in cortex (amyloid-rich) and cerebellum (amyloid-poor) of wild-type, *ABCA1* BAC, APP/PS1, and ABCA1/APP/PS1 mice using quantitative RT-PCR. Graphs are expressed as fold difference of all groups relative to wild-type controls, which were assigned an arbitrary value of 1. Data represent at least duplicate measurements for six to eight independent mice in each group. C: ABCA1 protein levels were evaluated by Western blot and normalized to GAPDH as an internal loading control from eight or more independent mice per genotype for each group, analyzed by one-way ANOVA. Data represent at least duplicate measurements per mouse. WT, wild type. Values shown are means ± SEM.

nificant difference in ABCA1 protein abundance in cortex or cerebellum was observed in any group ($P > 0.05$, n ⩾ 4 mice/genotype) (Fig. 6B), consistent with the mRNA results.

These data show that ABCA1 mRNA clearly is not induced in mice before amyloid deposition. However, we were surprised that *abca1* mRNA levels were not increased in the cortex of APP/PS1 mice at 12.7 months of age, because we had previously observed significantly increased *abca1* mRNA levels in APP/PS1 (APP/BAC−) cortex at 14.5 months of age, when each cohort was compared with littermate controls appropriately matched for age as well as genetic background. Notably, the difference in ages of these two cohorts is statistically significant ($P = 0.002$), and Aβ levels increase steeply in the APP/PS1 model over the age ranges used in these experiments. Therefore, it is possible that ABCA1 is induced only upon reaching a threshold level of Aβ that occurs between 12 and 14 months of age. Alternatively, it is possible that genetic background also may affect the response of ABCA1 expression to amyloid pathology.

To determine whether Aβ levels were indeed higher in the cohort with increased *abca1* mRNA levels, we directly compared guanidine-extractable Aβ levels in cortex and cerebellum of 14.5 month old APP/PS1 (APP/BAC−) mice on the 25% C3H, 75% C57Bl/6 background (referred to as group A or APP/BAC−) with those in 12.7 month old APP/PS1 animals on the 50% C3H, 50% C57Bl/6 background (referred to as group B or APP/PS1) (**Fig. 7**). Neither group for this analysis contained the human *ABCA1* BAC transgene. Cerebellum was examined because it was negative for any changes in ABCA1 expression in all of the experiments described above, even

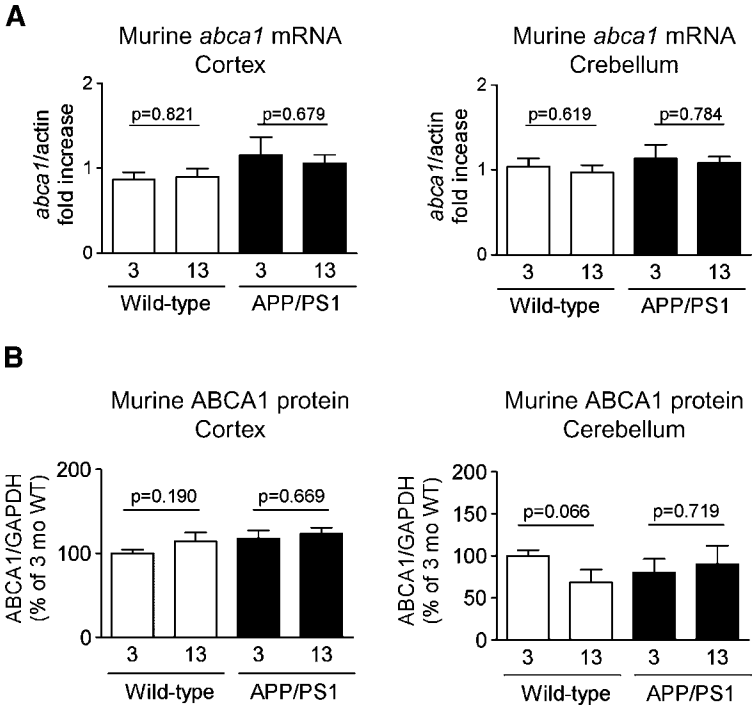

Fig. 6. ABCA1 mRNA is not increased in baseline APP/PS1 brains at ~3 (2.7 ± 0.243) or ~13 (12.7 ± 0.713) months of age. A: Murine *abca1* mRNA levels were determined in cortex and cerebellum using quantitative RT-PCR in 3- and 13-month old mice (n = 6–7 mice/group). Graphs are expressed as fold difference of all groups relative to wild-type controls at 3 months of age. B: ABCA1 protein levels were evaluated by Western blot and normalized to GAPDH as an internal loading control from four to six mice per group, analyzed by Student's *t*-test. Data represent at least duplicate measurements per mouse, expressed relative to 3 month old wild-type (WT) mice that were arbitrarily assigned an ABCA1 protein level of 100%. Values shown are means ± SEM.

though the presence of the PS1ΔE9 mutation in the APP/PS1 model of AD is associated with Aβ and amyloid accumulation in the cerebellum. Thioflavine-S staining revealed that cerebellar amyloid deposits were present but not as extensive as in cortex (data not shown). Aβ40 levels were significantly higher in group A or APP/BAC− than in group B or APP/PS1 animals for both cortex ($P = 0.038$, n ⩾ 6) and cerebellum ($P = 0.037$, n ⩾ 6). Furthermore, cortex contained significantly more Aβ40 than cerebellum for both group A or APP/BAC− ($P < 0.0001$, n ⩾ 6) and group B or APP/PS1 ($P = 0.003$, n ⩾ 6) mice. Similarly, Aβ42 levels were significantly higher in group A or APP/BAC− than in group B or APP/PS1 animals in cortex ($P = 0.039$, n ⩾ 6) and cerebellum ($P = 0.012$, n ⩾ 6), and cortex contained significantly more Aβ42 than cerebellum for both group A (APP/BAC−) ($P < 0.0001$, n ⩾ 6) and group B (APP/PS1) ($P = 0.0004$, n ⩾ 6) mice. These results demonstrate that even modest differences in age and/or genetic background significantly influence Aβ burden in the APP/PS1 model. More importantly, because *abca1* mRNA levels were increased only in cortex of animals with the highest Aβ levels (group A or APP/BAC− mice, 14.5 months of age, on a 25% C3H, 75% C57Bl/6 background), our results suggest that ABCA1 mRNA may be induced only in response to very advanced amyloid deposition.

Soluble apoE levels are increased in mice with amyloid deposits

Because transcription of ABCA1 is clearly not impaired in the amyloid-containing brain, posttranscriptional regulatory mechanisms may impose an upper limit on ABCA1 protein levels achievable in aged APP/PS1 mice with extensive amyloid deposits. Wang et al. (30) previously reported that apolipoproteins, including apoA-I and apoE3, stabilize ABCA1 protein in primary macrophages, hepatocytes, and transfected HEK cells. To determine whether apoE levels may be limiting in the amyloid-containing brain, we measured PBS-soluble and guanidine-insoluble apoE levels in wild-type, *ABCA1* BAC, APP/PS1, and ABCA1/APP/PS1 mice. Consistent with our previous observations (19), soluble apoE levels were increased ~2-fold in the amyloid-containing brain, and the presence of the *ABCA1* BAC did not affect this increase (n = 10–13, $P < 0.001$) (Figs. 1 and 2). These data show that soluble apoE is present in excess in the amyloid-containing brain, suggesting that mechanisms other than apoE availability must contribute

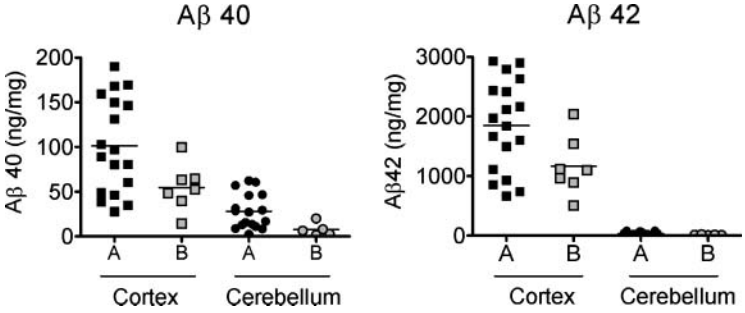

Fig. 7. Aβ40 and Aβ42 levels are significantly influenced by age and/or genetic background. Guanidine-extractable Aβ40 (A) and Aβ42 (B) levels were determined by ELISA in cohorts of APP/PS1 mice on a 25% C3H, 75% C57Bl/6 background at 14.5 months of age (group A) compared with cohorts of APP/PS1 mice on a 50% C3H, 50% C57Bl/6 background at 12.7 months of age (group B). Each data point represents an individual mouse (group A cortex, n = 18; group B cortex, n = 7; group A cerebellum, n = 17; group B cerebellum, n = 5).

to the failure to observe increased ABCA1 protein levels in brain regions rich in amyloid deposits.

DISCUSSION

ABCA1 is a critical regulator of apoE metabolism in the CNS (8, 9). We and others have demonstrated that deficiency of ABCA1 reduces the levels and lipidation of apoE, which results in enhanced amyloidogenesis in vivo (19–21). These findings raise the important question of whether overexpression of ABCA1 can increase apoE levels or lipidation and reduce amyloid burden. As a first step toward addressing this question, we crossed *ABCA1* BAC Tg mice to the APP/PS1 model of AD and analyzed the impact of human *ABCA1* on amyloid deposition, Aβ levels, and apoE abundance. We specifically selected the *ABCA1* BAC Tg model because it expresses human *ABCA1* in a physiologically accurate manner (22, 24) that has been shown to be atheroprotective in vivo (25, 26). Furthermore, we confirmed that, in mice without amyloid deposits, *ABCA1* BAC Tg mice exhibit a subtle 50% increase in cortical ABCA1 protein and a very modest 15% increase in cortical apoE protein compared with wild-type controls. These data show that BAC RP11-32H03 supports a slight but significant overexpression of ABCA1 in brain.

However, the presence of the *ABCA1* BAC made no significant impact on amyloid burden, Aβ levels, apoE abundance, or CSF cholesterol levels in aged APP/PS1 mice, raising the question of whether the BAC transgene remained functional in mice with amyloid deposits. Quantitative RT-PCR experiments demonstrated that human *ABCA1* mRNA was clearly present in ABCA1/APP/PS1 and not in APP/PS1 cortex, as expected. However, in amyloid-containing cortex, BAC RP11-32H03 did not make a significant contribution to the total ABCA1 mRNA pool; therefore, no increase in ABCA1 protein levels was detected. This is not attributable to a loss of global BAC transgene expression, because, in these same animals, human *ABCA1* constituted a significant proportion of total ABCA1 mRNA in liver and resulted in the expected increase in liver ABCA1 protein levels in ABCA1/APP/PS1 mice compared with APP/PS1 controls.

Further analysis revealed that ABCA1 expression appears to be responsive to local signals that may be generated in brain regions containing extensive amyloid deposits. Both human and murine ABCA1 mRNA levels were increased in cortex but not in cerebellum of aged ABCA1/APP/PS1 mice, demonstrating regional specificity in the ABCA1 transcriptional response. However, this local induction of ABCA1 mRNA does not necessarily lead to increased protein levels, as ABCA1/APP/PS1 mice, which have the highest levels of ABCA1 mRNA, did not display a proportional increase in cortical ABCA1 protein. In these cohorts, a very subtle increase in cortical ABCA1 protein was observed in the presence of the BAC transgene alone or in mice containing amyloid deposits, but these levels were not increased additively in mice with the BAC transgene as well as amyloid deposits. These observations suggest that ABCA1 may also be subject to posttranscriptional regulatory mechanisms that may impose an upper limit on the degree to which physiologically regulated ABCA1 protein levels can be reached in brain regions that are prominently affected in AD.

Furthermore, induction of ABCA1 transcription was evident only in mice with the highest Aβ and amyloid burdens. ABCA1 mRNA levels were not increased before amyloid deposition or in cohorts that contained abundant Aβ and amyloid load at 12.7 months of age. In contrast, a separate cohort that was slightly older (14.5 months of age) and had a 2-fold increase in Aβ40 and Aβ42 levels did display a significant increase in ABCA1 transcription, although we cannot rule out the possibility that genetic background may also significantly influence ABCA1 expression. Even though we cannot distinguish between the effects of genetic composition and age in this analysis, our results clearly demonstrate that physiologically regulated ABCA1 mRNA is induced only in advanced disease.

The mechanisms underlying this complex regulation of ABCA1 expression in the amyloid-containing brain remain to be completely defined. Transcription of ABCA1 is regulated by LXR/RXR agonists in both peripheral and CNS cells (17, 18, 31). Two different subtypes of LXRs, LXRα and LXRβ, have been described (32). LXRα is expressed at low levels in the CNS (33), whereas LXRβ is expressed broadly and at high levels in rodent brain (34–37). Among LXRβ ligands, 24*S*-hydroxycholesterol (24S-OH-Chol) and 27-hydroxycholesterol (27-OH-Chol) have been implicated in AD (38–40), and both are reported to induce ABCA1 transcription (18, 41). 24S-OH-Chol is the major cholesterol metabolite in brain and the primary route of cholesterol egress from the CNS (42, 43). 24S-OH-Chol is synthesized in the brain by cholesterol 24-hydroxylase, which in humans is expressed nearly exclusively in neurons (40, 42, 44, 45). In contrast, mice and rats generate only 50% and 70% 24S-OH-Chol in the brain, respectively (46, 47). 24S-OH-Chol levels in plasma and CSF are reported to be increased in subjects with mild to moderate AD (48–50), reflecting increased brain cholesterol turnover during the early stages of AD, when neurodegeneration is beginning. In severe to end-stage AD, however, extensive neuronal loss results in an eventual decline in 24S-OH-Chol levels in brain, CSF, and plasma (38, 40, 51). In contrast, 27-OH-Chol is produced in most peripheral cells (52), and the level of 27-OH-Chol in brain tissue is normally so low that it is useful as a marker of increased blood-brain barrier permeability and sterol influx into the CNS. Notably, the levels of 27-OH-Chol have been reported to be increased in postmortem human AD tissue and in the APP23 model of AD at ages concomitant with amyloid deposition (40), consistent with reports of blood-brain barrier leakiness as an early feature of AD pathogenesis (53). However, no differences were observed in brain 24S-OH-Chol levels in aging APP23 mice (54), suggesting either that changes during AD pathogenesis may be too subtle to detect in mice that have a broader cholesterol 24-hydroxylase distribution than humans or that the lack of extensive neurodegeneration in many models of

AD may result in little net change in 24-OH-Chol. Additional studies will be required to elucidate whether these or other oxysterols may be involved in the transcriptional response of ABCA1 to amyloid deposits.

ABCA1 expression is also regulated at the posttranscriptional level. In cholesterol-loaded macrophages, ABCA1 is degraded by the proteasomal system in a process that requires a functional Niemann-Pick type C gene (55). This pathway may be relevant during neurodegeneration, when increased free cholesterol is released from dying neurons and scavenged by microglia. In addition, both unsaturated and saturated fatty acids regulate ABCA1 protein stability in vitro (56, 57). ABCA1 contains a PEST sequence from amino acids 1,283–1,306 that modulates protein degradation by calpain (30). Residues tyrosine 1,286 and tyrosine 1,305 within the PEST sequence are normally constitutively phosphorylated, which results in a rapid turnover of ABCA1 in the absence of apolipoproteins. These residues become dephosphorylated in the presence of apoA-I or apoE, which stabilize ABCA1 by inhibiting calpain-mediated degradation (30, 58). We considered the possibility that limited amounts of soluble apoE, the major apolipoprotein in brain, may contribute to the lack of ABCA1 protein accumulation when ABCA1 mRNA levels are high. However, here we show that soluble apoE levels are in fact increased 2-fold in cortex with extensive amyloid deposits. This observation suggests that mechanisms other than apoE availability must contribute to the failure to accumulate high levels of ABCA1 protein in the amyloid-containing cortex.

Our findings pose a potential challenge to the consideration of using selective ABCA1 overexpression as a therapeutic means to increase apoE levels or lipidation in brains expected to develop AD neuropathology. Although we show that excess physiologically regulated ABCA1 can result in increased brain apoE levels, this was only observed in animals without amyloid deposits. It remains to be determined whether driving selective ABCA1 expression to much higher levels in the brain, for example using exogenous nonphysiological promoters, can override the regulatory mechanisms that appear to attenuate the expression of ABCA1 protein in the amyloid-containing brain and whether this will lead to increases in apoE levels and lipidation that will be sustained in the presence of AD neuropathology. Our study clearly demonstrates that physiologically regulated excess ABCA1 is unable to mitigate AD neuropathology in mice and suggests for the first time that ABCA1 may also be subject to transcriptional and posttranscriptional regulation during the pathogenesis of AD.

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